#8

Patent
Case No.: HA224

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: E.R. Squibb & Sons, Inc.
U.S.Patent No.:4,337,201
Issue Date: June 29, 1982
For: Phosphinylalkanoyl Substituted Prolines
Inventor: Edward W. Petrillo, Jr.

Princeton, New Jersey 08543-4000

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156

Hon. Commissioner of Patents and Trademarks:

Washington, D.C. 20231

Sir:

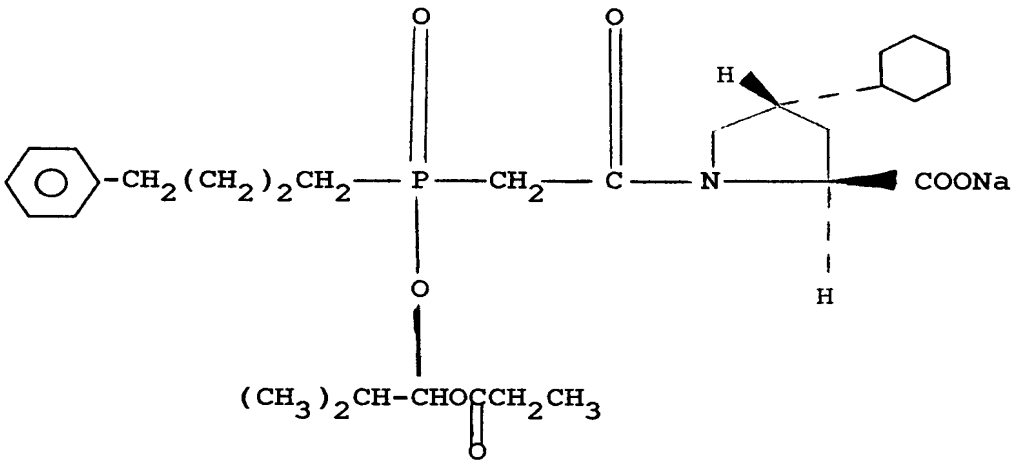
In accordance with the provisions of 35 U.S.C. 156, E. R. Squibb & Sons, Inc., a corporation of the state of Delaware, having a place of business at Lawrenceville-Princeton Road, Princeton, New Jersey 08540 hereby applies for an extension of 2 years of the term of United States Patent No. 4,337,201 issued June 29, 1982.

The following items are relevant and follow the guidelines set forth by the United States Patent and Trademark Office Rules of Practice; 37 CFR §1.710, et seq.

1) This application for extension is based upon the regulatory review period before the Food and Drug Administration of Monopril®. Monopril® is the trademark of E.R. Squibb & Sons, Inc. for an angiotensin converting enzyme inhibitor

product having as its active ingredient fosinopril sodium. The package insert for Monopril® is enclosed herewith.

Fosinopril sodium is designated chemically as L-proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy]-(4-phenylbutyl)phosphinyl]acetyl], sodium salt, trans and has the following structure



2) Regulatory review of Monopril® occurred under Section 505 of the Federal Food, Drug and Cosmetic Act (21 USC 355).

3) Monopril® received permission for commercial marketing and use under Section 505 of the Federal Food, Drug and Cosmetic Act on May 16, 1991.

4) Fosinopril sodium is the only active ingredient in Monopril®. Fosinopril sodium has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act.

5) This application for extension of the term of United States Patent No. 4,337,201 is being submitted within the 60 day period permitted for submission pursuant to 37 CFR §1.720(f) beginning on May 16, 1991. The last day on which the application could be submitted is July 15, 1991.

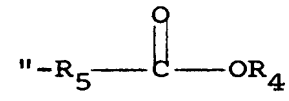
6) This application for extension of patent term seeks to extend the term of United States Patent No. 4,337,201 issued June 29, 1982, which unless extended will expire on June 29, 1999. This patent has not previously been extended. The inventor named in the patent is Edward W. Petrillo, Jr. The application is assigned to E.R. Squibb & Sons, Inc. by an assignment recorded on April 11, 1982 in the United States Patent and Trademark Office at Reel 3958, Frame 194.

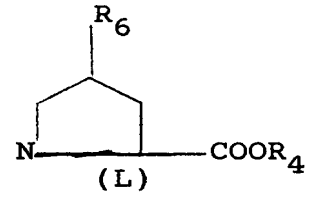
7) Attached hereto is a copy of United States Patent 4,337,201.

8) No disclaimers, certificates of correction, maintenance fees or reexamination certificates have been filed or issued in United States Patent No. 4,337,201.

9) United States Patent No. 4,337,201 claims fosinopril sodium, the active ingredient in Monopril®. The package insert for Monopril® shows that it is in tablet form. Monopril® is approved in tablet strengths of 10 mg/tablet and 20 mg/tablet.

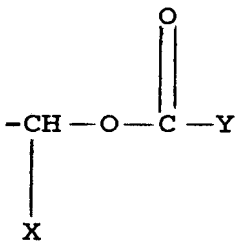
Claims 1, 2, 3, 4, and 7 as allowed in United States Patent No. 4,337,201 each includes fosinopril sodium within its scope. Note in particular the structural formula set out in Claim 1.

In Claim 1, "  "

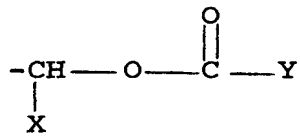
"  "

can be ,

R_4 can be "hydrogen" and it is specified that salts are included, R_6 can be "cycloalkyl" (defined to include groups having 3 to 7 carbon atoms), R_3 can be "hydrogen", n can be "zero", R_1 can be "arylalkyl" (aryl is defined to include phenyl and alkyl is defined to include groups having 1 to 10 carbon atoms), and

R_2 can be "  " and

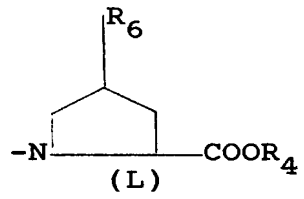
X and Y are both defined to include alkyl (alkyl is defined to include groups having 1 to 10 carbon atoms). Thus, Claim 1 covers salts of fosinopril and covers fosinopril sodium.

In Claim 2, which is dependent on Claim 1, one of R_2 and R_4 is defined as "hydrogen" and the other is defined as "  " Thus, for the reasons set forth in the discussion of Claim 1, Claim 2 covers salts of fosinopril and covers fosinopril sodium.

Claim 4 is dependent on Claim 1 and specifies that n is "zero". For the reasons as set forth in the above discussion of Claim 1, Claim 4 also covers salts of fosinopril and thus covers fosinopril sodium.

Claim 3 is dependent on Claim 4 and specifies that R_1 is "4-phenylbutyl" and R_3 is "hydrogen". For the reasons set forth in the above discussion of Claims 1 and 4, Claim 3 covers salts of fosinopril and thus covers fosinopril sodium.

Claim 7 is dependent on Claim 1 and specifies that " $-R_5-COOR_4$ " is

"  "

For the reasons set forth in the above discussion of Claim 1, Claim 7 also covers salts of fosinopril and thus covers fosinopril sodium.

10) The relevant dates and information pursuant to 35 USC 156(g) that will enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

For 35 USC 156(g) (1) (B) (i)-

The Notice of Claimed Investigational Exemption For A New Drug (IND number 23,103) for Monopril®, under the provisions of Section 505(i) of the Federal Food, Drug and Cosmetic Act, was filed on November 14, 1983, and became effective thirty days later on December 14, 1983.

The New Drug Application (number 19-915) for Monopril®, under Section 505(b) of the Federal Food, Drug and Cosmetic Act, was filed on November 15, 1988.

For 35 USC 156(g) (1) (B) (ii)-

The New Drug Application (number 19-915) for Monopril®, under Section 505(b) of the Federal Food, Drug and Cosmetic Act, was filed on November 15, 1988.

The New Drug Application (number 19-915) for Monopril®, under Section 505(b) of the Federal Food, Drug and Cosmetic Act, was approved on May 16, 1991.

11) The following is a brief description of certain significant activities undertaken by E. R. Squibb & Sons, Inc. during the applicable regulatory review period with respect to Monopril® including the dates applicable to such activities. Numerous other activities occurred which are not being listed.

November 14, 1983	- Investigational New Drug Application 23,103 was filed. This provided for initial clinical studies under protocol No. 23,103-2.
December 14, 1983	- The first shipment of clinical supplies in the United States.
December 15, 1983	- The first use in humans in the United States.

Continuing from this date forth, through the time of FDA approval, there were clinical studies in progress and/or being planned, with regular and frequent communications between E. R. Squibb and Sons, Inc. and the FDA, and between E. R. Squibb and Sons, Inc. and its clinical investigators.

December 3, 1984	- Submission of a progress report.
December 14, 1984	- Submission of an additional protocol.
November 27, 1985	- Submission of a progress report.

January 22, 1986	-	Submission of an additional protocol.
March 31, 1986	-	Submission of an additional protocol.
May 22, 1986	-	Submission of an additional protocol.
June 11, 1986	-	Submission of a drug experience report.
September 5, 1986	-	Submission of an additional protocol.
September 10, 1986	-	Submission of an additional protocol.
March 26, 1987	-	Submission of an additional protocol.
April 2, 1987	-	Submission of an additional protocol.
May 7, 1987	-	Submission of an additional protocol.
June 11, 1987	-	Submission of non-clinical and clinical reports.
June 22, 1987	-	Submission of an addtional protocol.
August 19, 1987	-	Submission regarding packaging of fosinopril sodium.
September 1, 1987	-	Specifications and test methods of IND are updated.
October 9, 1987	-	Submission of IND safety report.
December 21, 1987	-	Submission of IND safety report.
April 6, 1988	-	Submission of IND annual report.
April 26, 1988	-	Submission of an additional protocol.
August 31, 1988	-	Submission of IND safety report.
September 15, 1988	-	Submission of an additional protocol.

October 7, 1988	-	Submission of an additional protocol.
November 10, 1988	-	Submission of IND safety report.
November 15, 1988	-	Submission of New Drug Application 19-915 for Monopril®.
March 13, 1989	-	Submission of IND annual report.
March 17, 1989	-	Four month safety update provided to FDA.
April 14, 1989	-	Samples of fosinopril sodium provided to FDA laboratories for methods validation.
April 25, 1989	-	Submission of an additional protocol.
April 26, 1989	-	Submission of an additional protocol.
April 26, 1989	-	Submission of an additional protocol.
May 11, 1989	-	Submission of bioavailability studies.
May 19, 1989	-	Submission of supplemental analysis of four month safety update.
May 19, 1991	-	Submission of a response to an FDA letter requesting additional information on formulations.
November 30, 1989	-	Response submitted to FDA letter regarding manufacturing and control section of fosinopril sodium NDA.
December 21, 1989	-	Submission of an additional protocol.
February 22, 1990	-	Submission of revised draft package insert incorporating new safety data.
March 7, 1990	-	Submission of draft of pre-clinical section of the "Summary Basis of Approval".
May 2, 1990	-	Submission of draft of clinical section of the "Summary Basis of Approval".

May 2, 1990	- Submission of revised draft package insert.
June 7, 1990	- Submission of safety update.
July 27, 1990	- Submission of response regarding points raised by the FDA relating to carcinogenicity studies.
October 11, 1990	- Meeting with FDA to discuss carcinogenicity issues.
November 7, 1990	- Submission of information supporting proposed dosing schedule.
November 27, 1990	- Submission of safety update.
January 29, 1991	- Submission of an additional protocol.
March 20, 1991	- Submission of final printed bottle and carton labels and a revised draft package insert.
May 1, 1991	- Submission of safety update.
May 3, 1991	- Submission of final package insert.
May 16, 1991	- New Drug Application for Monopril® approved by FDA.

12) It is the opinion of E. R. Squibb & Sons, Inc. that United States No. 4,337,201 is eligible for a two year extension of its term. This two year extension period is the regulatory review period for Monopril® computed in accordance with 35 USC 156(g) (1) (B) (i) and (ii) which is 2710 days reduced by one-half the period described in 35 USC 156(g) (1) (B) (i) (i.e., 899 days) which is then limited by the provisions of 35 USC 156(g) (6) (c) so as not to exceed two years.

13) E. R. Squibb & Sons, Inc. and the undersigned acknowledge a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information that is material to the determination of entitlement to the extension sought.

14) Authorization is given to charge the Six Hundred Dollar ($600.00) fee for receiving and acting upon the application for extension to Deposit Account No. 19-3880. In the event the actual fee differs from this amount it is requested that the overpayment or underpayment be credited or charged to Deposit Account No. 19-3880.

15) The name, address, and telephone number of the person to whom inquiries and correspondence relating to this application for patent term extension should be directed is:

Donald J. Barrack
Bristol-Myers Squibb Company
P.O. Box 4000
Princeton, New Jersey 08543-4000
609-921-4328

16) A duplicate copy of this application, certified as such, is enclosed.

17) A signed declaration by a representative of E. R. Squibb & Sons, Inc. is submitted herewith in compliance with 37 CFR 1.740(a) (17).

Respectfully submitted,

Date: May 30, 1991

Donald J. Barrack
Registration No. 26,414

DJB/kac

Patent
Case No.: HA224

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: E. R. Squibb & Sons, Inc.
U.S.Patent No.:4,337,201
Issue Date: June 29, 1982
For: Phosphinylalkanoyl Substituted Prolines
Inventor: Edward W. Petrillo, Jr.

Princeton, New Jersey 08543-4000

DECLARATION

Hon. Commissioner of Patents and Trademarks:

Washington, D.C. 20231

I, Donald J. Barrack, residing at East Brunswick, New Jersey, declare as follows:

1. That I am an assistant secretary of E. R. Squibb and Sons, Inc., a corporation of the state of Delaware, having a place of business at Lawrenceville-Princeton Road, Lawrenceville, New Jersey, I am an attorney registered to practice in the United States Patent and Trademark Office under registration no. 26,414 and I have general authority from E. R. Squibb and Sons, Inc. to act on its behalf in patent matters.

2. That E. R. Squibb & Sons, Inc. is the assignee of the entire right, title and interest in United States Patent 4,337,201 by assignment recorded in the United States Patent and Trademark Office on April 11, 1982 at Reel 3958, Frame 194.

3. That I have reviewed and understand the contents of the Application For Extension Of Patent Term Under 35 USC 156 for United States Patent No. 4,337,201 which is submitted herewith.

4. That I believe that the above-identified patent is subject to an extension pursuant to 37 CFR §1.710.

5. That I believe that a 2 year extension of the term of the patent is fully justified under 35 USC 156 and the applicable regulations.

6. That I believe that the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 CFR §1.720.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application for extension of patent term and the validity of United States Patent No. 4,337,201.

Date: May 30, 1991

Donald J. Barrack
Registration No. 26,414

United States Patent [19]

Petrillo, Jr.

[11] **4,337,201**

[45] **Jun. 29, 1982**

[54] **PHOSPHINYLALKANOYL SUBSTITUTED PROLINES**

[75] Inventor: **Edward W. Petrillo, Jr., Pennington, N.J.**

[73] Assignee: **E. R. Squibb & Sons, Inc., Princeton, N.J.**

[21] Appl. No.: **212,911**

[22] Filed: **Dec. 4, 1980**

[51] **Int. Cl.**[3] **A61K 31/40; C07D 207/12**

[52] **U.S. Cl.** **548/413;** 424/274; 424/246; 424/250; 424/251

[58] **Field of Search** 260/326.2, 326.36, 326.35, 260/326.47, 326.42, 326.46

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,105,775	8/1978	Ondetti et al.	424/274
4,154,935	5/1979	Ondetti et al.	260/326.2
4,168,267	9/1979	Petrillo, Jr.	260/326.2
4,217,359	8/1980	Krapcho	260/326.2
4,234,489	11/1980	Ondetti et al.	260/326.25

FOREIGN PATENT DOCUMENTS

2027025	2/1980	United Kingdom	260/326.2
2028327	3/1980	United Kingdom	260/326.2

OTHER PUBLICATIONS

Chemistry and Industry, Jun. 7, 1980, pp. 433–462.

Primary Examiner—Jane T. Fan
Attorney, Agent, or Firm—Lawrence S. Levinson; Donald J. Barrack

[57] **ABSTRACT**

Esters of phosphinylalkanoyl prolines and phosphinylalkanoyl substituted prolines are inhibitors of angiotensin converting enzyme and are useful in the treatment of hypertension.

13 Claims, No Drawings

PHOSPHINYLALKANOYL SUBSTITUTED PROLINES

BACKGROUND OF THE INVENTION

The recent literature discloses a variety of mercaptoacyl amino acids which are useful for inhibiting the conversion of angiotensin I to angiotensin II in mammals, and are, therefore, useful in the treatment of hypertension. U.S. Pat. No. 4,105,776, issued Aug. 8, 1978 discloses mercaptoacyl amino acids wherein the amino acid is, inter alia, proline, 4-hydroxyproline and 4-alkylproline.

U.S. Pat. No. 4,154,935, issued May 15, 1979 discloses mercaptoacyl amino acids wherein the amino acid is, inter alia, 4-halogen substituted proline or 4,4-dehalogen substituted proline.

United Kingdom patent application No. 2,027,025, published Feb. 13, 1980, discloses mercaptoacyl amino acids wherein the amino acid is 5-substituted prolines.

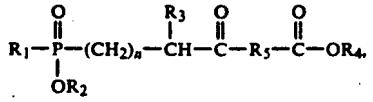
United Kingdom patent application No. 2,028,327, published Mar. 5, 1980, discloses mercaptoacyl amino acids wherein the amino acid is, inter alia, proline substituted in the 3- or 4-position with with a group having the formula R—S— or R—O— wherein R is alkyl, alkenyl, alkynyl, phenyl, substituted phenyl, phenylalkyl or substituted phenylalkyl.

U.S. Pat. No. 4,168,267, issued Sept. 18, 1979 discloses phosphinylalkanoyl prolines and esters or salts thereof.

The compounds disclosed by the above mentioned references are disclosed as inhibitors of the action of angiotensin converting enzyme in mammals and as useful hypotensive agents.

BRIEF DESCRIPTION OF THE INVENTION

Compounds having the formula

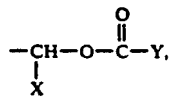


I

and salts thereof, have hypotensive activity. In formula I, and throughout the specification, the symbols are as defined below.

R_1 is alkyl, aryl, arylalkyl, cycloalkyl, or cycloalkyl (alkyl);

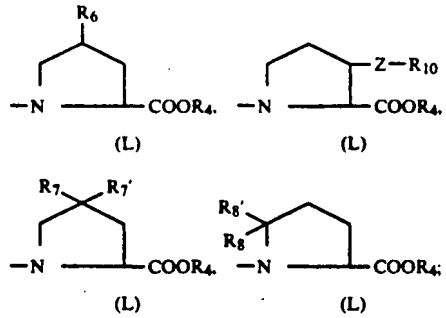
R_2 and R_4 each is independently hydrogen, alkyl, arylalkyl or



wherein X is hydrogen, alkyl, or phenyl and Y is hydrogen, alkyl, phenyl or alkoxy, or together X and Y are $-(CH_2)_2-$, $-(CH_2)_3-$, $-CH=CH-$, or

R_3 is hydrogen or alkyl;

$-R_5-COOR_4$ is



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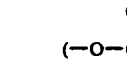
R_6 is hydrogen, hydroxy, alkyl, halogen, azido, amino, cycloalkyl, aryl, arylalkyl, carbamoyloxy



N,N-dialkylcarbamoyloxy, or $-Z-R_9$;

R_7 and R_7' are the same and each is halogen or $-Z-R_{10}$, or R_7 and R_7' together are $=O$, $-O-(CH_2)_m-O-$ or $-S-(CH_2)_m-S-$;

R_8 is hydrogen and R_8' is phenyl, 2-hydroxyphenyl or 4-hydroxyphenyl or R_8 and R_8' together are $=O$;

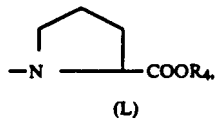
R_9 is alkyl, aryl, arylalkyl, 1- or 2-naphthyl, or biphenyl;

R_{10} is alkyl, aryl or arylalkyl;

Z is oxygen or sulfur;

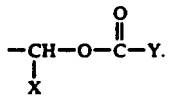
n is 0 or 1; and

m is 1 or 2; with the proviso that if $-R_5-COOR_4$ is



(L)

at least one of R_2 and R_4 is



The term "aryl", as used throughout the specification either by itself or as part of a larger group, refers to phenyl or phenyl substituted with halogen, alkyl, alkoxy, alkylthio, hydroxy, alkanoyl, nitro, amino, dialkylamino, or trifluoromethyl groups. Phenyl and monosubstituted phenyl are preferred and phenyl is the most preferred.

The term "alkyl", as used throughout the specification eith?r by itself or as part of a larger group, refers to groups having 1 to 10 carbon atoms. Alkyl groups having 1 to 4 carbon atoms are preferred.

The term "cycloalkyl", as used throughout the specification either by itself or as part of a larger group, refers to groups having 3 to 7 carbon atoms.

The term "alkoxy", as used throughout the specification either by itself or as part of a larger group, refers to groups having 1 to 8 carbon atoms. Alkoxy groups having 1 to 3 carbon atoms are preferred.

The term "halogen", as used throughout the specification either by itself or as part of a larger group, refers to fluorine, chlorine, bromine and iodine. The preferred halogen groups are fluorine and chlorine.

The term "alkanoyl", as used throughout the specification either by itself or as part of a larger group, refers to groups having 2 to 9 carbon atoms.

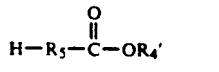
DETAILED DESCRIPTION OF THE INVENTION

The compounds of formula I, and salts thereof, are hypotensive agents. They inhibit the conversion of the decapeptide angiotensin I to angiotensin II and, therefore, are useful in reducing or relieving angiotensin related hypertension. The action of the enzyme renin on angiotensinogen, a pseudoglobulin in blood plasma, produces angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II. The latter is an active pressor substance which has been implicated as the causative agent in several forms of hypertension in various mammalian species, e.g., humans. The compounds of this invention intervene in the angiotensinogen→(renin)→angiotensin I→(ACE)→angiotensin II sequence by inhibiting angiotensin converting enzyme and reducing or eliminating the formation of the pressor substance angiotensin II. Thus by the administration of a composition containing one (or a combination) of the compounds of this invention, angiotensin dependent hypertension in a species of mammal (e.g., humans) suffering therefrom is alleviated. A single dose, or preferably two to four divided daily doses, provided on a basis of about 0.1 to 100 mg. per kilogram of body weight per day, preferably about 1 to 15 mg. per kilogram of body weight per day is appropriate to reduce blood pressure. The substance is preferably administered orally, but parenteral routes such as the subcutaneous, intramuscular, intravenous or intraperitoneal routes can also be employed.

The compounds of this invention can also be formulated in combination with a diuretic for the treatment of hypertension. A combination product comprising a compound of this invention and a diuretic can be administered in an effective amount which comprises a total daily dosage of about 30 to 600 mg., preferably about 30 to 330 mg. of a compound of this invention, and about 15 to 300 mg., preferably about 15 to 200 mg. of the diuretic, to a mammalian species in need thereof. Exemplary of the diuretics contemplated for use in combination with a peptide of this invention are the thiazide diuretics, e.g., chlorthiazide, hydrochlorthiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methychlothiazide, trichlormethiazide, polythiazide or benzthiazide as well as ethacrynic acid, ticrynafen, chlorthalidone, furosemide, musolimine, bumetanide, triamterene, amiloride and spironolactone and salts of such compounds.

The compounds of formula I can be formulated for use in the reduction of blood pressure in compositions such as tablets, capsules or elixirs for oral administration, or in sterile solutions or suspensions for parenteral administration. About 10 to 500 mg. of a compound of formula I is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is such that a suitable dosage in the range indicated is obtained.

The phosphinylalkanoyl substituted prolines of formula I can be prepared by reacting a proline derivative have the formula

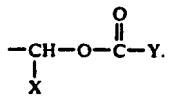


II

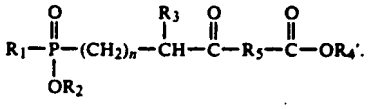
with a phosphinyl-acetic or propionic acid having the formula

III

In formula II and throughout the specification, R_4' is alkyl, arylalkyl or

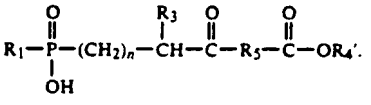


The reaction can be accomplished using known amide bond forming procedures. For example, the reaction can be run in the presence of a coupling agent such as dicyclohexylcarbodiimide, or the acid of formula III can be activated by formation of its mixed anhydride, symmetrical anhydride, acid halide (preferably acid chloride) or acid ester, or by the use of Woodward reagent K, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, N,N'-carbonyldiimidazole or the like. A review of these methods can be found in *Methoden der Organischen Chemie* (Houben-Weyl), Vol. XV, part II, page 1 et seq. (1974). The product of the reaction has the formula



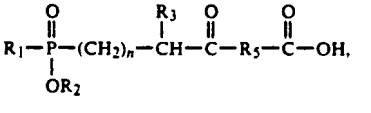
IV

Compounds of formula I wherein R_2 is hydrogen can alternatively be obtained by (i) treating a corresponding compound of formula IV wherein R_2 is alkyl with a halosilane such as bromotrimethylsilane and then water or (ii) catalytic hydrogenation of a corresponding compound of formula IV wherein R_2 is arylalkyl, e.g., using palladium on charcoal. These products have the formula



V

Compounds of formula I wherein R_4 is hydrogen, i.e., compounds having the formula

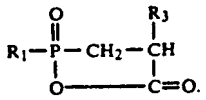


VI

can be obtained by basic hydrolysis of a compound of formula IV or V. Alternatively, a compound of formula

IV or V wherein R_4' is an easily removable ester group (such as t-butyl) can be treated with trifluoroacetic acid and anisole to obtain the carboxylic acids of formula I.

The phosphinylalkanoyl substituted prolines of formula I wherein n is 1 can alternatively be prepared by reacting a proline derivative of formula II with a phospholane having the formula



VII

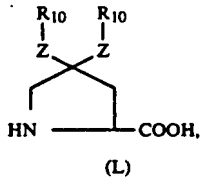
The reaction proceeds most readily when run in the presence of an organic base, e.g., triethylamine, pyridine, N,N-dimethylamine or the like, in an inert organic solvent such as acetonitrile, dichloromethane, ether, tetrahydrofuran, or the like.

Phosphinyl-acetic or propionic acid derivatives of formula III can be prepared using known procedures; see, for example, U.S. Pat. No. 4,168,267, issued Sept. 18, 1979. Phospholanes of formula VII can be prepared following the procedures described in Zh. Obsh. Kim., 37:411 (1967) and Zh. Obsh. Kim., 38:288 (1968).

The proline esters of formula II are known or are readily obtainable using known esterification techniques which are illustrated in the examples. Various substituted prolines are disclosed by Manger et al., Chem. Rev., 66:47 (1966). Ondetti et al. disclose various alkyl, halogen, ether and thioether substituted prolines in U.S. Pat. Nos. 4,105,776, 4,154,935, and U.K. Application No. 2,028,327. Iwao et al. in U.K. Application No. 2,027,025 disclose various 5-substituted prolines.

As disclosed by Krapcho in U.S. Ser. No. 66,119, filed Aug. 12, 1979, the carbamoyloxy substituted prolines can be obtained by reacting the hydroxy substituted N-protected proline with phosgene and then a dialkylamine. Removal of the N-protecting group yields the desired starting material.

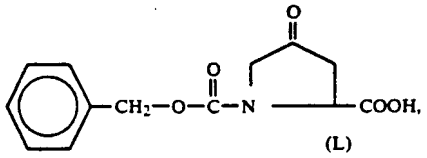
As disclosed by Krapcho in U.S. Ser. No. 99,164, filed Nov. 30, 1979, the prolines of the formula



VIII

(L)

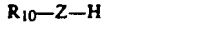
and esters thereof, can be prepared by reacting a keto compound of the formula



IX

(L)

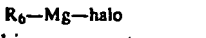
or ester thereof, with an alcohol or thiol having the formula



X

in the presence of an orthoformate or thioformate of the formula $HC(Z—R_{10})_3$ and an acid such as concentrated sulfuric acid or p-toluenesulfonic acid. Removal of the carbobenzyloxy group by catalytic hydrogenation when Z is oxygen or by treatment with hydrogen bromide and acetic acid when Z is sulfur yields the desired compound.

As disclosed by Krapcho in U.S. Ser. No. 164,985, filed Aug. 7, 1980, the 4-substituted proline starting materials wherein the substituent R_4 is cycloalkyl, aryl, or arylalkyl can be prepared by reacting a 4-keto proline of formula IX, or ester thereof, with a solution of Grignard reagent

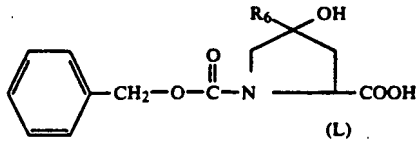


XI

or lithium reagent

XII

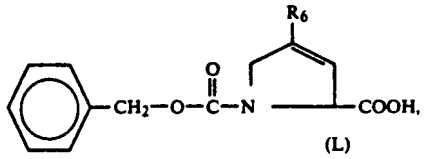
to yield a compound of the formula



XIII

(L)

or ester thereof. This compound can be treated with a dehydrating agent such as p-toluenesulfonic acid, sulfuric acid, potassium bisulfate, or trifluoroacetic acid to yield a 3,4-dehydro-4-substituted proline having the formula

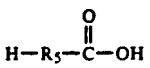


XIV

(L)

or ester thereof. Removal of the N-benzyloxycarbonyl protecting group and hydrogenation of the resulting compound yields the desired 4-substituted proline derivatives. The substituted proline wherein R_6 is cyclohexyl can also be prepared by further hydrogenation of the 4-phenylproline compound.

Additional processes for preparing the compounds of this invention will be apparent to the practitioner of this invention. For example, the carboxyl group of a proline derivative having the formula

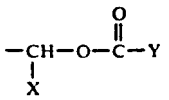


XV

can be protected, e.g., by conversion to an amine salt, or a 2-hydroxyethyl or diphenylmethyl ester, reacted with a phosphinyl-acetic or propionic acid of formula III, and then deprotected to yield a product of formula VI.

Esterification of a product of formula VI using art-recognized procedures yields the corresponding product of formula IV.

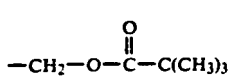
An alternative procedure for preparing the compounds of this invention wherein R_2 is alkyl, arylalkyl or



and R_4 is hydrogen comprises first alkylating the corresponding compound of formula V and then subjecting the resulting compound to basic hydrolysis.

The practitioner of this invention will also appreciate that the phosphinylalkanoyl-4-aminoprolines of this invention can be prepared from the corresponding phosphinylalkanoyl-4-azidoprolines and the phosphinylalkanoyl-4-azidoprolines can be prepared from the corresponding phosphinylalkanoyl-4-hydroxyprolines.

The compounds of this invention wherein one of R_2 and R_4 is



(i.e., pivaloyloxymethyl) and the other is hydrogen are preferred esters of this invention.

The compounds of this invention wherein at least one of R_2 or R_4 is hydrogen, form basic salts with various inorganic and organic bases which are also within the scope of the invention. Such salts include ammonium salts, alkali metal salts like lithium, sodium and potassium salts (which are preferred), alkaline earth metal salts like the calcium and magnesium salts, salts with organic bases, e.g., dicyclohexylamine salt, benzathine, N-methyl-D-glucamine, hydrabamine salts, salts with amino acids like arginine, lysine and the like. The nontoxic, physiologically acceptable salts are preferred, although other salts are also useful, e.g., in isolating or purifying the product. The salts are formed using conventional techniques.

The following examples are specific embodiments of this invention.

EXAMPLE 1

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, methyl ester

[Ethoxy(4-phenylbutyl)phosphinyl]acetic acid (2.42 g), acetonitrile (25 ml) and carbonyldiimidazole (1.32 g) are allowed to stir under argon at 0° C. for 1 hour. 4,4-Ethylenedithioproline, methyl ester (1.48 g) is added to the mixture, which is then allowed to stir at room temperature for about 16 hours. The solvent is stripped off leaving an oil that is diluted with ethyl acetate (100 ml), washed with 5% potassium bisulfate, washed with saturated sodium bicarbonate and filtered through anhydrous sodium sulfate. The solvent is stripped off yielding the title compound as an oil (3.44 g).

EXAMPLE 2

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, methyl ester

(S)-7-[[Ethoxy(4-phenylbutyl)-phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, methyl ester (3.44 g) and dry dichloromethane (15 ml) are added to a flask under an argon atmosphere. Using a gas-tight syringe, bromotrimethylsilane (1.5 ml) is added to the flask, which is stirred for about 16 hours at room temperature. Ethyl acetate (50 ml) and water (10 ml) are added to the flask and stirred for 30 minutes. The mixture is diluted with ether and transferred to a separatory funnel. The organic layer is extracted with saturated sodium bicarbonate (two 25 ml portions) and the extract is acidified to pH 1.0 to precipitate product which is extracted into ethyl acetate. The ethyl acetate extract is washed with brine, run through sodium sulfate to dry, and the solvent is stripped leaving the title compound as an oil (2.99 g).

EXAMPLE 3

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, methyl ester (2.99 g) and 1 N sodium hydroxide (15 ml) is stirred for 30 minutes at room temperature. The reaction mixture is extracted with ether (50 ml) and the aqueous phase is acidified with concentrated hydrochloric acid to pH 1.0. A solid precipitates out of solution along with an oil. This is extracted into ethyl acetate, passed through sodium sulfate, and the solvent is stripped yielding 2.74 g of the title compound, melting point 105.5°–107° C.

EXAMPLE 4

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4.]nonane-8-carboxylic acid, dilithium salt

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid (2.74 g) in 1 N lithium hydroxide (10.6 ml) is passed through a column of 80 ml of ion-exchange resin (AG50WX8 (Li^+)). The fractions containing product are filtered through a millipore filter and most of the solvent is stripped off. The eluate is lyophilized to give 2.53 g of the title compound as a powder. The product contains 2 moles of water and has an optical rotation $[\alpha]_D = -13.2°$ (c = 13.5 mg/ml methanol).

EXAMPLE 5

(S)-4-Hydroxy-1-[[ethoxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline, methyl ester

A mixture of [ethoxy(4-phenylbutyl)phosphinyl]acetic acid (1.71 g), acetonitrile and carbonyldiimidazole (0.97 g) is stirred under argon at 0° C. for 1 hour. (S)-4-hydroxyproline, methyl ester (0.006 mole) is suspended in acetonitrile, added to the mixture and stirred at room temperature 90 minutes. Solvent is stripped off, and the resulting oil is taken up in ethyl acetate. washed with 5% potassium bisulfate, washed with saturated sodium bicarbonate, washed with brine. dried over magnesium sulfate, and the solvent stripped to leave 2.23 g of the title compound as an oil.

EXAMPLE 6

(S)-4-Azido-1-[[ethoxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline, methyl ester

(S)-4-Hydroxy-1-[[ethoxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline, methyl ester (2.0 g), triphenylphosphine (2.57 g), 2.1 N hydrazoic acid/benzene (12 ml, >5 equivalents) and dry acetonitrile are stirred under argon at room temperature for 30 minutes. Diethylazocarboxylate (1.71 g) in acetonitrile (5 ml) is

added dropwise over 30 minutes. After 18 hours the reaction is still incomplete (as shown by thin-layer chromatography) and triphenylphosphine (1 equivalent), diethylazocarboxylate (1 equivalent) and 2.1 N hydrazoic acid (6 ml) are added. After 4 hours the reaction is complete. Nitrogen is bubbled through the solution to remove excess hydrazoic acid and the solvent is stripped leaving an oil. The oil is chromatographed on 100 mg of silica gel, eluting first with ethyl acetate and then with 3% methanol/ethyl acetate to give 1.64 g of the title compound.

EXAMPLE 7

(S)-4-Azido-1-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline, methyl ester

(S)-4-Azido-1-[[ethoxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline, methyl ester (1.60 g), dry dichloromethane and bromotrimethylsilane (0.85 g) are stirred under argon at room temperature. After 19 hours, an additional equivalent of bromotrimethylsilane is added and stirring is continued for 2 hours. Excess bromotrimethylsilane and dichloromethane is removed in vacuo and the residue is treated with ethyl acetate/water and stirred for 15 minutes. The layers are separated and the ethyl acetate layer is extracted with saturated sodium bicarbonate. The aqueous extracts are acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate. The extract is washed with water, brine, dried over sodium sulfate, and the solvent stripped to yield 1.35 g of the title compound as an oil.

EXAMPLE 8

(S)-4-Azido-1-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline

(S)-4-Azido-1-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline, methyl ester (1.30 g) and 1 N sodium hydroxide (about 15 ml) is stirred for 30 minutes. The reaction mixture is acidified with concentrated hydrochloric acid, extracted with ethyl acetate, washed with water, washed with brine, dried over sodium sulfate, and the solvent is stripped yielding 1.11 g of the title compound having an optical rotation $[\alpha]_D = -22.3°$ (c = 10 mg/ml, methanol). The product contains ½ mole of water.

EXAMPLE 9

(S)-4-Amino-1-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline

A mixture of (S)-4-azido-1-[hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-L-proline (0.8 g) acetic acid (about 20 ml) and 10% palladium on charcoal is hydrogenated on a Parr apparatus for 3 hours. The reaction mixture is filtered through Celite to remove the catalyst and the solvent is stripped leaving an oil. The oil is diluted with water and washed several times with ethyl acetate. The aqueous phase is lyophilized yielding 0.48 g of crude product as a solid. Crude product (400 mg) is run on a column of ion-exchange resin AG50X8 (about 30 ml), eluting first with water and then with a pyridine-acetic acid buffer (pH 6.5). The fractions containing the desired material are combined and evaporated leaving a glass, which is dissolved in water and lyophilized yielding 300 mg of the title compound having an optical rotation $[\alpha]_D = -24.8°$ (c = 10 mg/ml, methanol).

EXAMPLE 10

(R)-1-[[Ethoxy(2-phenylethyl)phosphinyl]acetyl-4-hydroxy-L-proline,methyl ester

[Ethoxy(2-phenylethyl)phosphinyl]acetic acid (1.27 g) and carbonyldiimidazole (0.8 g) are stirred in 25 ml of acetonitrile at 0° C. for 1 hour. trans-4-Hydroxy-L-proline, methyl ester hydrochloride (1.0 g) is shaken with triethylamine (0.76 ml) in 10 ml of acetonitrile and filtered directly into the reaction mixture. After standing under nitrogen for 6 days at room temperature, the mixture is taken up in ethyl acetate (500 ml) and 5% sodium bisulfate (10 ml). The organic layer is washed with brine (10 ml), followed by saturated sodium bicarbonate, a second brine washing, and then dried over sodium sulfate and evaporated. The residue, 1.8 g, is crystallized from toluene yielding 0.6 g of the title compound, melting point 126°–128° C.

EXAMPLE 11

(S)-7-[[Hydroxy(2-phenylethyl)phosphinyl]acetyl]-1,4-dioxa-7-azaspiro[4.4]nonane-8-carboxylic acid, ammonia salt

(A)

(S)-7-[[Benzyloxy(2-phenylethyl)phosphinyl]acetyl]-1,4-dioxa-7-azaspiro[4.4]nonane-8-carboxylic acid, 2-hydroxyethyl ester

Carbonyldiimidazole (2.04 g) is added to a cooled solution (0° C.) of benzyloxy(2-phenylethyl)phosphinylacetic acid (4.0 g) in acetonitrile (50 ml) and the mixture is stirred for 1 hour. A solution of 1,4-dioxa-7-azaspiro[4.4]nonane-8-carboxylic acid, 2-hydroxyethyl ester is added and stirred for 1 hour at 0° C. and for about 16 hours at room temperature. The mixture is taken up in ether, washed with 5% potassium busulfate, followed by brine, saturated sodium bicarbonate and two additional brine washes, and then dried over sodium sulfate and evaporated to give 5.6 g of an oil. The oil is flash chromatographed on silica gel eluted with ethyl acetate and ethyl acetate/methanol (9:1) yielding 3.5 g of the title compound as an oil.

(B)

(S)-7-[[Hydroxy(2-phenylethyl)phosphinyl]acetyl]-1,4-dioxa-7-azaspiro[4.4]nonane-8-carboxylic acid, ammonia salt

(S)-7-[[Benzyloxy(2-phenylethyl)phosphinyl]acetyl]-1,4-dioxa-7-azaspiro[4.4]nonane-8-carboxylic acid, 2-hydroxyethyl ester (3.3 g) is dissolved in 50 ml of methanol, and lithium hydroxide monohydrate (0.54 g) dissolved in a small amount of water is added. The mixture is stirred for 1 hour at room temperature and hydrogenated over 1 g of 10% palladium on charcoal at 1 atmosphere for 3 hours. The mixture is stirred for 1 hour at room temperature and hydrogenated over 1 g of 10% palladium on charcoal at 1 atmosphere for 3 hours. The mixture is filtered through Celite, the filtrate evaporated to dryness and the residue dissolved in water which is filtered through a Millipore filter and lyophilized yielding 2.84 g of material. Chromatography of this material on 150 ml of Sephadex (OH form, pH 7.4) eluted with a gradient buffer (0.005 M to 0.5 M ammonium bicarbonate). Fractions which are pure (electrophoresis) are combined, evaporated and lyophilized from water (twice) yielding 1.1 g of the title compound having an optical rotation $[\alpha]_D = -25.9°$ (c = 1, water).

EXAMPLE 12

1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

Hydroxy(4-phenylbutyl)phosphinyl acetic acid (51.2 g) is suspended in 300 ml of dichloromethane and 1 ml of dimethylformamide in a magnetically-stirred 1000 ml three-neck flask with thermometer and condenser. The mixture is cooled to 5° C. with an ice bath and thionyl chloride (16.1 ml) is added. The bath is removed and the mixture is stirred at room temperature for forty-five minutes. A heating mantle is fitted and the mixture is refluxed for seventy-five minutes. Steady evolution of gas continues until the end of the reflux period, when it stops. The flask is cooled to 5° C. and a solution of proline pivaloyloxymethyl ester tosylate (86.0 g) in 300 ml of dichloromethane (previously dried with 10 g of molecular sieves) is added. Triethylamine (85 ml) is added, causing a rise in temperature to 25° C. The mixture is stirred for about 16 hours and then washed with three 100 ml portions of 1 N HCl, then brine, and evaporated to a residue (106.6 g). The residue is dissolved in 400 ml of acetone and added dropwise through a needle to 2000 ml of water containing seed crystal and vigorously stirred in a 5000 ml flask. The product precipitates as a powder which is filtered immediately and dried at first in vacuo (1 mm, dry ice trap), then over silica gel to yield 86.5 g of the title compound, melting point 63°–66° C.

Anal. Calc'd. for $C_{23}H_{35}NO_7PH_2O$: C, 56.90; H, 7.47; N, 2.89; P, 6.38. Found: C, 57.02; H, 7.35; N, 2.94; P, 6.4.

EXAMPLE 13

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester

(A)

(S)-7-[(1,1-Dimethylethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester

To a mixture of 7.0 g of 1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, hydrochloride, 17.4 ml of water and 12.1 ml of triethylamine under argon at room temperature is added a solution of 7.8 g of 2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile in 17.4 ml of dioxane. After 3 hours the reaction mixture is diluted with water and washed twice with ether. The aqueous portion is acidified with concentrated hydrochloric acid and the resulting oil is extracted into ethyl acetate. The ethyl acetate layer is washed with water and brine, dried over magnesium sulfate, and the solvent is stripped yielding an oil. The oil crystallizes on standing yielding 8.9 g of material.

The above material is dissolved in dimethylformamide, treated with (3.7 g) and chloromethyl pivalate (5.2 ml) and the resulting mixture is stirred at room temperature for 16 hours under argon. The reaction mixture is partitioned between water and ethyl acetate, and the organic phase is washed with saturated sodium bicarbonate, water, brine and dried ($MgSO_4$). The solvent is stripped to obtain an oil (10.3 g). The crude product is chromatographed on silica (200 g) eluting with hexene/ether (3/1) to give 8.55 g of the title compound as crystalline solid, melting point 79.5°–81° C. The product is triturated with cold ligroin then filtered to obtain the pure product.

Analysis calc'd for $C_{18}H_{19}NO_6S_2$: C, 51.53; H, 6.97 N, 3.34; S, 15.28. Found: C, 51.34; H, 6.90; N, 3.32; S, 14.87.

(B)

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester

A mixture of 1.7 g of [ethoxy(4-phenylbutyl)phosphinyl]acetic acid, acetonitrile and 0.96 g of carbonyldiimidazole is stirred under argon at 0° C. for 1 hour. (S)-7-[(1,1-Dimethylethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester (2.5 g) is treated with trifluoroacetic acid (about 2 ml) and stirred at room temperature for 30 minutes. The trifluoroacetic acid is removed in vacuo, the residue taken up in acetonitrile and added dropwise to the above mixture over a 20 minute period at room temperature. After an additional 2.5 hours, the acetonitrile is stripped and the resulting oil is partitioned between ethyl acetate and water. The layers are separated and the organic portion is washed with 5% potassium sulfate, saturated sodium bicarbonate, brine, dried ($MgSO_4$) and evaporated. The residue (3.4 g) is chromatographed on silica (120 g) eluting with ethyl acetate/dichloromethane (1:1) and yielding 2.7 g of the title compound as a glass.

EXAMPLE 14

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester (2.7 g) in dichloromethane (dried over alumina) is treated with bromotrimethylsilane (1.2 ml) via syringe and the reaction mixture is stirred for 16 hours under argon. The solvent and excess bromotrimethylsilane is evaporated in vacuo and the residue is taken up in ethyl acetate and water and stirred for 15 minutes. The layers are separated and the ethyl acetate is washed with 5% potassium sulfate, water, brine dried ($MgSO_4$) and evaporated. The 2.3 g of crude product is chromatographed on silica (75 g) eluting with dichloromethane/methanol/acetic acid (19/0.5/0.5). After stripping solvent 1.9 g of product is left as a glass.

EXAMPLE 15

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy) methyl ester (1.82 g) is dissolved in 75% water/acetone and treated with 0.1208 g of lithium carbonate. Additional water is added and after 15 minutes the solvent is stripped. The resulting gel is dissolved in water and filtered. The filtrate is run through a Millipore filter and lyophilized to obtain 1.54 g of the product as a solid.

Analysis calc'd for $C_{25}H_{35}NO_7S_2P^-Li^+.1.5$ mole H_2O: C, 50.83; N, 2.37; H, 6.48; S, 10.86; P, 5.2. Found: C, 51.07; N, 2.36; H, 6.19; S, 10.80; P, 5.5.

EXAMPLE 16

1-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline, benzyl ester

Triethylamine (1.9 ml) and chloromethyl pivalate (2.0 ml) are added to a solution of 3.0 g of [[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline, benzyl ester in dimethylformamide under an argon atmosphere and the resulting mixture is stirred at room temperature for 6 hours. The reaction mixture is diluted with ethyl acetate, washed with water, brine, dried ($MgSO_4$), and evaporated. The crude product (3.7 g) is chromatographed on silica (80 g) eluting with ethyl acetate/dichloromethane to give 2.96 g of the title compound.

EXAMPLE 17

1-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline

To a solution of 2.6 g of 1-[[[2,2-dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline, benzyl ester is added 250 mg of 10% palladium on charcoal and the resulting mixture is shaken in a Parr hydrogenation apparatus for 2.5 hours. The catalyst is filtered through a Celite bed and the methanol is stripped from the filtrate. The crude product (2.0 g) is chromatographed on silica (50 g), eluting with dichloromethane/acetic acid/methanol (19:0.5:0.5). The solvent is stripped and the remaining acetic acid is azeotroped off with toluene to yield 1.91 g of the title compound as a glass.

EXAMPLE 18

1-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline, lithium salt

1-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline (1.067 g) is dissolved in 50% acetone/water. Lithium carbonate (0.083 g) is added to the stirring solution. After 30 minutes the acetone and water is removed in vacuo to leave a clear oil. The oil is dissolved in water, run through a Millipore filter and lyophilized to yield 1.0 g of a granular solid.

Analysis of $C_{23}H_{33}NO_7P-Li^+$.1 mole H_2O: Found: C, 56.28; N, 2.80; H, 7.19; P, 6.2. Calc'd: C, 56.21; N, 2.85; H, 7.18; P, 6.3.

EXAMPLE 19

(S)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(4-fluorophenoxy)-L-proline

[Ethoxy(4-phenylbutyl)phosphinyl]acetic acid (2.53 g) is dissolved in 30 ml of acetonitrile and cooled to 0° C. in an ice-bath. Carbonyldiimidazole (1.58 g) is added under argon and the resulting mixture is stirred for 1 hour at 0° C. A second solution of 4-(4-fluorophenoxy)-L-proline (2.0 g) is prepared in 20 ml of dry acetonitrile, bis-(trimethylsilyl)acetamide (1.81 g) is added and the mixture is stirred under argon for about 1 hour. The second solution is added to the first and the mixture is stirred for about 16 hours at room temperature. Concentration of the mixture in vacuo yields an oily semisolid which is taken up in 200 ml of dichloromethane, washed with 5% potassium bisulfate, saturated sodium bicarbonate, saturated sodium chloride, and the dichloromethane solution dried over anhydrous magnesium sulfate. The material (3.73 g) is flash chromatographed (20% acetic acid-benzene) to yield 1.36 g of the title compound.

EXAMPLE 20

(S)-4-(4-Fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-L-proline

(S)-4-(4-Fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline (0.380 g) and dry dichloromethane (10 ml) are added to a flask under an argon atmosphere at room temperature. Then bis-(trimethylsilyl)acetamide (0.157 g) is added and the mixture is allowed to stir for ½ hour. Bromotrimethylsilane (0.112 ml) is added to the mixture which is stirred for about 16 hours at room temperature. A few drops of water are added to the reaction mixture and stirring is continued for an additional ½ hour. The solvent is stripped off in vacuo yielding 0.372 g of solid. This material is taken up in saturated sodium bicarbonate (15 ml) and washed with ether (two 30 ml portions); this is acidified to pH 2.0 with concentrated hydrochloric acid. The mixture is extracted with ethyl acetate (three 25 ml portions). The ethyl acetate extracts are combined and washed with saturated brine (50 ml) followed by drying over anhydrous $MgSO_4$, and the solvent is then removed leaving 0.110 g of the title compound.

EXAMPLE 21

(S)-4-(4-Fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-L-proline, dilithium salt

(S)-4-(4-Fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)phsophinyl]acetyl]-L-proline (0.1 g) in 1.0 N lithium hydroxide (0.37 ml) is passed through a column of 20 ml of ion-exchange resin (Li^+). The fractions containing product are passed through a Millipore filter and most of the solvent is stripped off in vacuo. The eluate is lyophilized and then heated for 24 hours under vacuum at 100° C. to yield 0.017 g of the title compound as a dihydrate, melting point >250° C.

Analysis calc'd for $C_{23}H_{25}NFPO_6Li_2.2H_2O$: C, 54.02; H, 4.97; N, 2.74; F, 3.72; P, 6.06. Found: C, 53.91; H, 5.06; N, 2.72; F, 3.73; P, 6.30.

EXAMPLE 22

(cis)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

(A)

(cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-(phenylmethyl)-L-proline

To a solution of cis-4-benzyl-L-proline hydrochloride (3 g) and triethylamine (5 g) in water (10 ml) is added a solution of 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (3.5 g) in dioxane (10 ml). The mixture is stirred at ambient temperature for 4 hours. After the addition of water (30 ml), and washing with ether, the mixture is acidified to a pH of 3–4 with 10% citric acid. The oil that separates is extracted into ethyl acetate, washed with brine, and dried ($MgSO_4$). After concentration in vacuo, the residue (3.7 g) solidifies at room temperature, melting point 145°–149° C. dec.

(B)
(cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

To a solution of (cis)-1-[(1,1-dimethylethoxy)carbonyl]-4-(phenylmethyl)-L-proline (3.4 g) in dry dimethylformamide (3.0 ml) is added solid potassium fluoride (1.5 g), followed by chloromethyl pivalate (2.0 g). After stirring for 16 hours at ambient temperature the mixture is diluted with water (100 ml) and extracted with ethyl acetate. The ethyl acetate solution is washed with 5% sodium bicarbonate, water, brine, and dried ($MgSO_4$). The solvent is removed in vacuo to give an oil residue (5 g). The oil is chromatographed on silica gel (120 g) eluting with ether/hexane (1:3) to give the product (4.4 g) as an oil that solidifies on standing, melting point 60°–62° C.

(C)
(cis)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

A mixture of (cis)-1-[(1,1-dimethylethoxy)carbonyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester (4.0 g) and 10 ml of trifluoroacetic acid is stirred at combined temperature under argon for 45 minutes. The mixture is concentrated in vacuo at ambient temperature and the residue is dissolved in acetonitrile (40 ml). A second solution is prepared of [ethoxy(4-phenylbutyl)phosphinyl]acetic acid (2.7 g) in acetonitrile (20 ml). The solution is cooled to 0° C., treated with carbonyldiimidazole (1.5 g) and stirred for 1 hour. Triethylamine (1.0 g) is added and the cold bath is removed. The first solution is added dropwise to the second and the mixture is stirred at room temperature for 16 hours and concentrated in vacuo. The residue is dissolved in ethyl acetate (200 ml), washed with 5% potassium acid sulfate, saturated sodium bicarbonate, brine, and dried ($MgSO_4$). The solvent is removed in vacuo; the oil residue (5.6 g) is chromatographed on silica gel (200 g), eluting with dichloromethane/acetone (1:1) to give the product, (4.6 g).

EXAMPLE 23

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

To a solution of (cis)-1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester (4.5 g) in dichloromethane (50 ml) is added bromotrimethylsilane (2 g). The mixture is stirred at ambient temperature for 16 hours. After concentration in vacuo, the residue is treated with water (25 ml) and extracted into dichloromethane (350 ml), washed with brine, and dried ($MgSO_4$). The solvent is removed in vacuo to give a glass-like solid (4.2 g). The residue is chromatographed on silica gel (220 g) eluting with dichloromethane/methanol/acetic acid (19:1:1), to give the product (2.7 g).

EXAMPLE 24

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

To a solution of (cis)-1-[[hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-4-(phenylmethyl)-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester in acetone/water (80 ml) is added solid lithium carbonate (0.0023 mole). The mixture is stirred at ambient temperature; as the solid gradually dissolves, separation of some orange, polymeric material is observed, plus a trace of solid in suspension. The mixture is filtered after 2 hours, and the filtrate is concentrated in vacuo at ambient temperature. The residue is treated with double distilled water (200 ml) and extracted with ether. An emulsion results that is separated with difficulty. The aqueous layer is Millipore filtered and lyophilized to give the title compound (1.0 g).

Analysis calc'd for $C_{30}H_{39}NO_7P.Li.3H_2O$: C, 58.32; H, 6.36; N, 2.26; P, 5.01. Found: C, 58.15; H, 6.38; N, 2.26; P, 4.98.

EXAMPLE 25

1-[[(Ethoxy)octylphosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

1-[(1,1-Dimethylethoxy)carbonyl]-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester (3.2 g) and p-toluenesulfonic acid monohydrate (1.72 g) in ethyl acetate (about 50 ml) is treated with 10% palladium on charcoal (300 mg) and shaken on a Parr hydrogenation apparatus at 30 psi for 1 hour. The reaction mixture is filtered through a Celite bed and concentrated to a small volume. The residue is diluted with ether and seeded. The resulting white crystals are filtered and washed four times with ether.

A mixture of [(ethoxy)octylphosphinyl]acetic acid (2.2 g), carbonyldiimidazole (1.4 g), and acetonitrile is stirred under argon at 0° C. for 1 hour. The above p-toluenesulfonic acid salt in about 20 ml of acetonitrile is then added dropwise over a 45 minute period at room temperature. After 60 hours the acetonitrile is stripped and the resulting slurry is diluted with ethyl acetate and water. The layers are separated and the ethyl acetate portion is washed with 5% potassium bisulfate, saturated sodium bicarbonate, brine and dried ($MgSO_4$). The solvent is stripped leaving 3.2 g of the title compound as an oil.

EXAMPLE 26

1-[(Hydroxyoctylphosphinyl)acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

Bromotrimethylsilane (1.5 ml) is added to a flask containing 3.2 g of 1-[(ethoxyoctylphosphinyl)acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester and dry dichloromethane under argon at room temperature. After 16 hours excess bromotrimethylsilane and solvent are evaporated in vacuo. The resulting oil is diluted with ethyl acetate and water. After stirring for 5 minutes the layers are separated. The ethyl acetate layer is washed with saturated sodium bicarbonate, 5% potassium sulfate, brine and dried ($MgSO_4$). The solvent is stripped leaving 3.0 g of oil. The oil is chromatographed on silica (120 g) eluting with dichloromethane/methanol/acetic acid (8:1:1). The solvent is stripped and the remaining acetic acid is azeotroped with toluene leaving 1.7 g of the title compound as an oil.

EXAMPLE 27

1-[(Hydroxyoctylphosphinyl)acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

1-[(Hydroxyoctylphosphinyl)acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester (1.5848 g) is dissolved in 80% acetone/water and treated with lithium

carbonate (0.13 g). The resulting precipitate is filtered off and the acetone and most of the water is stripped. The resulting soapy solution is diluted with water, Millipore filtered and lyophilized to give a hygroscopic solid (1.3 g).

Anal. Calc'd. for $C_{21}H_{37}NO_7PLi.1.75H_2O$: C, 52.50; H, 7.76; N, 2.91; P, 6.4. Found: C, 52.11; H, 7.81; N, 2.66; P, 6.2.

EXAMPLE 28

(cis)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylthio)-L-proline,(2,2-dimethyl-1-oxopropoxy) methyl ester

(A)

(cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-(phenylthio)-L-proline

To a solution of (cis)-4-(phenylthio)-L-proline (5 g) and triethylamine (3.4 g) in water (13 ml), a solution of 2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile (6.0 g) in dioxane (13 ml) is added. The mixture is stirred at ambient temperature. After one hour, water (50 ml) is added, the mixture is washed with ethyl acetate and acidified to a pH of 3–4 with 10% citric acid. The oil that separates is extracted into ethyl acetate, washed with brine, and dried ($MgSO_4$). The residue is dissolved in saturated sodium bicarbonate (50 ml) plus water (600 ml). The alkaline solution is washed with ether and acidified to a pH of 4 with 10% citric acid. The oil that separates from solution is extracted into ethyl acetate, washed with brine, and dried ($MgSO_4$). The solvent is removed in vacuo to give the product as an oil (5.9 g) that gradually solidifies, melting point 110°–118° C.

(B)

(cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-(phenylthio)-L-proline,(2,2-dimethyl-1-oxopropoxy) methyl ester

Solid potassium fluoride (2.2 g) is added to a solution of (cis)-1-[(1,1-dimethylethoxy)carbonyl]-4-(phenylthio)-L-proline (5.5 g) in dry dimethylformamide (25 ml), followed by the addition of chloromethylpivalate (3.0 g). The mixture is stirred at room temperature for 20 hours. After the addition of water (40 ml), the mixture is extracted with ethyl acetate, washed with saturated sodium bicarbonate, brine, and dried ($MgSO_4$). The solvent is removed in vacuo and the oil residue (8 g) is chromatographed on silica gel (180 g), eluting with ether/hexane (1:2) to give the product (5 g) as an oil, which gradually solidifes to a waxy solid, melting point 83°–85° C.

(C)

(cis)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylthio)-L-proline,(2,2-dimethyl-1-oxopropoxy)-methyl ester, lithium salt

The mixture (A) is prepared by stirring a solution of (cis)-1-[(1,1-dimethylethoxy)carbonyl]-4-(phenylthio)-L-proline,(2,2-dimethyl-1-oxopropoxy) methyl ester (4.2 g) in trifluoroacetic acid (10 ml) at ambient temperature. The mixture is concentrated in vacuo at ambient temperature and the residue is dissolved in dry acetonitrile (40 ml). It is added dropwise to the following mixture (B): a solution of [ethoxy(4-phenylbutyl)phosphinyl]acetic acid in acetonitrile (20 ml) is cooled to 0° C. and carbonyldiimidazole (1.6 g) is added; after stirring at 0° C. for one hour, and immediately preceding the addition of (A). triethylamine (1.1 g) is added. Following the addition of (A) the bath is removed and the mixture is stirred at ambient temperature for 20 hours.

After concentration in vacuo, the residue was dissolved in ethyl acetate (200 ml), washed with 5% potassium acid sulfate, saturated sodium bicarbonate, brine, and dried ($MgSO_4$). The solvent is removed in vacuo. The oil residue (5.6 g) is chromatographed on silica gel (200 g) eluting with dichloromethane/acetone (9:1) to give the product as a viscous oil (3.1 g).

EXAMPLE 29

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylthio)-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

To a solution of (cis)-1-[[ethoxy(4-phenylbutyl)phosphinyl]-acetyl]-4-(phenylthio)-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester (3 g) in dichloromethane (30 ml) is added bromotrimethylsilane (1.2 g). The mixture is stirred at room temperature for 16 hours. After the addition of water (15 ml), saturated sodium bicarbonate (25 ml) is added, followed by water (500 ml) to effect solution. The aqueous alkaline solution is washed with ether and acidified to a pH of 3 with concentrated hydrochloric acid. The oil that separates from solution is extracted into ethyl acetate, washed with brine, and dried ($MgSO_4$). The solvent is removed in vacuo to give a residue of 2.5 g which is chromatographed on silica gel (120 g) eluting with dichloromethane/methanol/acetic acid (19:1:1) to give the product (1.8 g) as a glass-like solid.

EXAMPLE 30

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylthio)-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

A solution of lithium carbonate (50 ml) is added dropwise to a stirring solution of (cis)-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-(phenylthio)-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester (0.5756 g) in acetone (10 ml); as the stirring solution becomes turbid, the turbidity is clarified by the addition of acetone, so that the final volume of the reaction mixture is 120 ml. A trace of orange polymeric material that separates from solution is removed by filtration. The mixture is concentrated to a volume of 50 ml, and is Millipore filtered and lyophilized to give the title compound (0.6 g).

Analysis calc'd for $C_{29}H_{37}NO_7PS.Li.2.5H_2O$: C, 55.57; H, 5.95; N, 2.23; P, 4.94; S, 5.12. Found: C, 55.67; H, 6.06; N, 2.12; P, 4.80; S, 5.38.

EXAMPLE 31

(cis)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

(A)

(cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-methoxy-L-proline, (2.2-dimethyl-1-oxopropoxy)methyl ester

Conversion of 5.0 g (cis)-1-[(1,1-dimethylethoxy)carbonyl]-4-methoxy-L-proline, cyclohexylamine salt to the free acid using 50 ml of 10% $KHSO_4$ and extraction into ethyl acetate gives 3.9 g of material. A mixture of the free acid with 2.2 g of chloromethylpivalate and 1.7 g of anhydrous potassium fluoride in 20 ml of dimethylformamide is stirred under argon at room temperature for 16 hours. The solution is diluted with 50 ml of ethyl acetate and treated with 20 ml of water (twice), satu-

-rated sodium bicarbonate and brine. The organic fraction is dried and evaporated in vacuo. The residue is triturated with hexane before drying to give 4.3 g of the title compound, melting point 88°-90° C.

(B) 4-Methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, tosylate salt

A solution of 3.0 g of (cis)-1-[(1,1-dimethylethoxy)-carbonyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxo-propoxy)methyl ester and 1.5 g of p-toluenesulfonic acid in 50 ml of ethyl acetate is treated with 0.3 g of 10% palladium on charcoal and hydrogenated at 30 psi for one hour. The product, which precipitates during this procedure, is dissolved in acetone before filtration. The solvent is evaporated in vacuo to give an oil which gradually solidifies. The material is triturated with ethyl acetate and filtered to give 2.8 g of product, melting point 116°-118° C.

(C) (cis)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

A stirred solution of 1.9 g of [ethoxy(4-phenylbutyl)-phosphinyl]acetic acid in 25 ml of dry acetonitrile is cooled to 0° C. and treated with 1.1 g of carbonyl-diimidazole under argon. After 1 hour the solution is treated with 1.04 ml of triethylamine and the ice bath is removed. A second solution is prepared consisting of 2.8 g of 4-methoxy-L-proline, (2,2-dimethyl-1-oxo-propoxy)methyl ester, tosylate salt in 25 ml of dry ace-tonitrile and is added to the first solution. The resulting mixture is stirred at room temperature for 16 hours. After evaporation of the solvent in vacuo, the oil resi-due is dissolved in ethyl acetate and extracted with 5% potassium sulfate, saturated sodium bicarbonate and brine. The organic layer is dried and evaporated in vacuo to give 2.4 g of crude product. This material is combined with 1.1 g of crude product from another experiment and chromatographed using acetone/ethyl acetate (3:1) to give 2.9 g of product.

Analysis calc'd for $C_{26}H_{40}NPO_8.H_2O$: C, 57.44; H, 7.72; N, 2.57. Found: C, 57.87; H, 7.49; N, 2.47.

EXAMPLE 32

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

A solution of 2.9 g of (cis)-1-[[ethoxy(4-phenylbutyl)-phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester in 50 ml of dry dichloro-methane is treated with 1.0 g of bromotrimethylsilane at 0° C., then stirred at room temperature for 16 hours under argon. The solvent is evaporated in vacuo and the oil residue is dissolved in ether and treated with an excess of saturated sodium. The aqueous portion is treated with 6 N HCl to pH 1.5 and the product is ex-tracted into ethyl acetate, dried and evaporated in vacuo to give 1.4 g of product.

Analysis calc'd for $C_{24}H_{34}NO_8P.\frac{1}{2}H_2O$: C, 57.13; H, 6.99; N, 2.76. Found: C, 56.54; H, 7.98; N, 2.66.

EXAMPLE 33

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

A solution of 1.36 g of (cis)-1-[[hydroxy(4-phenyl-butyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester is dissolved in 4 ml of dioxane and cooled to 15° C. while adding 27.2 ml of 0.099 N lithium hydroxide. The temperature is re-duced to 5° C. during the addition procedure. Lyophili-zation gives 1.2 g of product, melting point 81°-85° C.

EXAMPLE 34

(trans)-1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

Following the procedure of Example 31, but starting with (trans)-1-[(1,1-dimethylethoxy)carbonyl]-4-methoxy-L-proline, cyclohexylamine salt, yields the title compound.

Analysis calc'd: $C_{26}H_{40}NO_8P.1.OH_2O$: C, 57.44; H, 7.79; N, 2.57; P, 5.70. Found: C, 57.07; H, 7.37; N, 2.75; P, 5.4.

EXAMPLE 35

(trans)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

Following the procedure of Example 32, but starting with (trans)-1-[[ethoxy(4-phenylbutyl)phosphinyl-]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxo-propoxy)methyl ester, yields the title compound.

Analysis calc'd: $C_{24}H_{36}NO_8P.0.5H_2O$: C, 56.90; H, 7.36; N, 2.77; P, 6.12. Found: C, 57.01; H, 7.39; N, 2.72; P, 5.70.

EXAMPLE 36

(trans)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

Following the procedure of Example 33, but starting with (trans)-1-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxo-propoxy)methyl ester, yields the title compound, $[\alpha]_D = -33.9°$, c = 10 mg/ml methanol.

EXAMPLE 37

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid

A mixture of 2.0 g of [ethoxy(4-phenylbutyl)phos-phinyl]acetic acid and 1.1 g of carbonyldiimidazole in acetonitrile is stirred under argon at 0° C. for 1 hour and then treated with 1.9 g of triethylamine and 1.7 g of 1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, hy-drochloride. After 4.5 hours the acetonitrile is stripped and the residue is partitioned between ethyl acetate and 1 N HCl. The organic phase is washed with brine, dried ($MgSO_4$), and the solvent is stripped to give 3.2 g of the title compound as an oil.

EXAMPLE 38

(S)-7-[[[(2.2-Dimethyl-1-oxopropoxy)methoxy]-(4-phenylbutyl)phosphinyl]acetyl]-1.4-dithia-7-azas-piro[4.4]nonane-8-carboxylic acid

(A) (S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, diphenylmethyl ester

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid (3.2 g) in

ethyl acetate is treated with 2.0 g of diphenyldiazomethane in ethyl acetate. After 3.5 hours, the reaction mixture is partitioned between ethyl acetate and water. The layers are separated and the organic portion is washed with saturated sodium bicarbonate, 5% potassium bisulfate, brine and dried ($MgSO_4$). The solvent is stripped leaving 6.1 g of residue which is chromatographed on 180 g of silica, eluting with ethyl acetate to yield 3.4 g of the title compound as an oil.

(B)

(S)-7-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, diphenylmethyl ester

(S)-7-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, diphenylmethyl ester (3.4 g) in dry dichloromethane is treated with bromotrimethylsilane (1.5 ml) under argon at room temperature. After 3 hours the dichloromethane and excess bromotrimethylsilane are removed in vacuo and the residue taken up in water and ethyl acetate and stirred for 5 minutes. The layers are separated and the organic phase is washed with brine and dried ($MgSO_4$). The solvent is stripped yielding 3.3 g of the title compound as a foam.

(C)

(S)-7-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, diphenylmethyl ester

Chloromethylpivalate (1.6 ml) is added to a stirred mixture of (S)-7-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-1,4-dithia-7-azaspiro[3.3]nonane-8-carboxylic acid, diphenylmethyl ester (3.3 g), triethylamine (1.5 ml) and dimethylformamide under argon at room temperature. After 16 hours an additional equivalent of triethylamine and chloromethylpivalate is added to the mixture. After an additional 24 hours, the mixture is partitioned between ethyl acetate and water. The layers are separated and the organic phase is washed with saturated sodium bicarbonate, 5% potassium bisulfate, brine and dried ($MgSO_4$). The solvent is stripped leaving 5.0 g of residue which is chromatographed on 150 g of silica gel, eluting with 33% hexane/ethyl acetate to yield 2.4 g of the title compound as an oil.

(D)

(S)-7-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy]-(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid

(S)-7-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, diphenylmethyl ester (2.4 g) in dry dichloromethane is treated with 3.0 ml of trifluoroacetic acid under argon at room temperature. After 1 hour, the trifluoroacetic acid and dichloromethane are removed in vacuo and the resulting oil partioned between ethyl acetate and water. The layers are separated and the organic phase is washed with brine, dried ($MgSO_4$) and evaporated. The residue (4.0 g) is chromatographed on 130 g of silica eluting with dichloromethane/acetic acid/methanol (18:1:1) to give (after azeotropic removal of acetic acid with toluene) 1.5 g of the title compound.

EXAMPLE 39

(S)-7-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy]-(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, lithium salt

(S)-7-[[[(2,2-Dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid (0.48 g) is dissolved in acetone and treated with lithium carbonate (0.0321 g) and water. The solution is stirred for 1.5 hours. The acetone and water are removed in vacuo and the resulting residue is taken up in water, Millipore filtered and lyophilized to give 0.48 g of the title compound.

Analysis for $C_{25}H_{35}NO_7S_2P-Li^+.1.5$ moles H_2O: Calc.: N, 2.37; C, 50.84; H, 6.48; S, 10.86; P, 5.24. Found: N, 2.42; C, 50.69; H, 6.02; S, 10.28; P, 5.06.

EXAMPLE 40

(trans)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline,dilithium salt

A solution of 650 mg of (trans)-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt in 15 ml of ethanol and 10 ml of water containing 6 ml of 1 N sodium hydroxide is stirred for 16 hours at room temperature. Ethanol is removed in vacuo and water was added. The aqueous layer is washed with ethyl acetate (discard), acidified with 8 ml of 1 N HCl and extracted twice with ethyl acetate. The organic layer is washed with saturated brine, combined, dried ($MgSO_4$) and concentrated in vacuo. The residue is heated in an oil bath at 75° C. under high vacuum for 2 hours. The residue (500 mg) is dissolved in 1.5 ml of 1 N lithium hyroxide and washed through a column of ion exchange resin (lithium form, 10 ml) with water. The organics containing eluates are combined, Millipore filtered and lyophilized for 16 hours to give 475 mg of product.

EXAMPLE 41

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline,dilithium salt

A solution of 200 ml of (cis)-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-methoxy-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester in 5 ml of ethanol and 5 ml of water containing 1.5 ml of 1 N sodium hydroxide is stirred for 16 hours at room temperature. Ethanol is removed in vacuo and water is added. The aqueous layer is washed with ethyl acetate (discard), acidified with 3 ml of 1 N HCl and extracted twice with ethyl acetate. The organic layer is washed with saturated brine, combined, dried ($MgSO_4$) and concentrated in vacuo. The residue is heated in an oil bath at 75° C. under high vacuum for 2 hours. The residue (ca. 150 mg) is dissolved in 0.4 ml of 1 N lithium hydroxide and washed through a column of ion exchange resin (lithium form, 5 ml) with water. The organics containing eluates are combined. Millipore filtered and lyophilized for 16 hours to give 120 mg of product.

EXAMPLE 42

1-[[Ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2.2-dimethyl-1-oxopropoxy)methyl ester

A solution of 2.4 g 1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline in acetone is treated with 445 mg of potassium carbonate dissolved in a small amount of water. Solvent is removed in vacuo and water is

azeotropically removed by addition of toluene and evaporation in vacuo several times. The potassium salt is suspended in fresh acetone and 1.03 g of chloromethylpivalate and 0.5 ml of 25% aqueous sodium iodide is added. The mixture is heated at reflux temperature for 3 hours and allowed to stir at room temperature for 16 hours. The solids are filtered and the filtrate is concentrated in vacuo. The residue (3.3 g) is dissolved in ethyl acetate, washed with 5% sodium bicarbonate, saturated brine, dried ($MgSO_4$), charcoaled and concentrated in vacuo to give 2.65 g of material. Flash chromatography with acetone elution gives 2.4 g of product.

Analysis calc'd for $C_{25}H_{38}NO_7P.1H_2O$: C, 58.46; H, 7.85; N, 2.73; P, 6.03. Found: C, 58.30; H, 7.24; N, 2.58; P, 5.98.

EXAMPLE 43

1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester, lithium salt

An ice cold aqueous dioxane solution of 704 mg of 1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2.2-dimethyl-1-oxopropoxy)methyl ester is treated with 1.57 ml of 0.96 N lithium hydroxide. Dioxane is removed in vacuo, additional water is added and the solution is lyophilized to give 700 mg of the title compound.

EXAMPLE 44

(cis)-4-(4-Fluorophenoxy)-1-[[ethoxy(4-phenylbutyl)-phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxo-propoxy)methyl ester

Following the procedure of example 42, but starting with (cis)-1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-4-(4-fluorophenoxy)-L-proline, yields the title compound.

EXAMPLE 45

(cis)-4-(Fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxo-propoxy)methyl ester

A solution of (cis)-4-(4-fluorophenoxy)-1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester (0.24 g) in dichloromethane (2.5 ml), to which bromotrimethylsilane (0.12 g) is added, is stirred at ambient temperature for 16 hours. The mixture is concentrated in vacuo and the residue is treated with water (7 ml). The oil that separates is extracted into ether; the ethereal solution is washed with brine and dried ($MgSO_4$). The solvent is removed in vacuo to give the product as a glass-like solid (0.219 g).

EXAMPLE 46

(cis)-4-(Fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-L-proline,(2.2-dimethyl-1-oxo-propoxy)methyl ester, lithium salt

An aqueous solution of lithium carbonate is added dropwise, with stirring, to a solution of (cis)-4-(fluorophenoxy)-1-[[hydroxy(4-phenylbutyl)phosphinyl-]acetyl]-L-proline,(2.2-dimethyl-1-oxopropoxy)methyl ester (0.17 g) in acetone (10 ml); as the stirring solution becomes turbid, the turbidity is clarified by the addition of acetone, so that the final volume of the reaction mixture is 30 ml. A trace of insolubles is removed by filtration. The filtrate is concentrated in vacuo to a volume of 10 ml. After the addition of water (15 ml), the solution is Millipore filtered and lyophilized to give 0.14 g of the title compound.

Analysis calc'd for $C_{29}H_{36}FNO_8P.Li.1.75H_2O$: C, 56.63; H, 6.18; N, 2.27; F, 3.09; P, 5.04. Found: C, 56.60; H, 6.15; N, 2.25; F, 2.80; P, 5.10.

EXAMPLE 47

(cis)-4-Cyclohexyl-1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline

(cis)-4-Cyclohexyl-L-proline hydrochloride (3.0 g) in 30 ml acetonitrile is treated with bis(trimethylsilyl-)acetonitrile (0.525 g) and stirred until the solid has dissolved. Meanwhile, [(ethoxy) (phenylbutyl)]phosphinyl acetic acid (4.0 g) and carbonyldiimidazole (3.87 g) in 70 ml of acetonitrile are stirred at 0° C. for one hour. The two solutions are combined and stirred for about 16 hours. The mixture is concentrated and the residue is taken up in dichloromethane, washed with 5% potassium bisulfate, and brine, dried ($MgSO_4$) and evaporated to an oil which is chromatographed (20% acetic acid/benzene) to yield 3.36 g of the title compound.

EXAMPLE 48

(cis)-4-Cyclohexyl -1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline

(cis)-4-Cyclohexyl-1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline, (0.83 g), bis(trimethylsilyl-)acetamide (0.47 ml) and bromotrimethylsilane (0.28 ml) are stirred in 20 ml of dichloromethane for about 16 hours. A small amount of water is added and the mixture is evaporated to an oil residue (0.61 g). This material is crystallized from acetone with a recovery of 0.047 g of solid, melting point 175°–176° C.

Anal. Calc'd. for $C_{23}H_{34}NO_5P$, MW 435.50: C, 63.43; H, 7.87; N, 3.22; P, 7.11. Found: C, 62.97; H, 7.93; N, 3.19; P, 7.0.

EXAMPLE 49

(cis)-4-Cyclohexyl-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)-methyl ester

(A)

(cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-cyclohexyl-L-proline,2,2-dimethyl-1-oxopropoxy)methyl ester

Following the procedure of example 13A, but substituting (cis)-4-cyclohexyl-L-proline hydrochloride for 1,4-dithio-7-azaspiro[4.4]nonane-8-carboxylic acid. hydrochloride, yields the title compound.

(B)

(cis)-4-Cyclohexyl-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)-methyl ester

A mixture of [hydroxy(4-phenylbutyl)phosphinyl]acetic acid (1.2 g) tetrahydrofuran and carbonyldiimidazole (0179 g) is stirred under argon at 0° C. for 1 hour. (cis)-1-[(1,1-Dimethylethoxy)carbonyl]-4-cyclohexyl-L-proline,(2,2-dimethyl-1-oxopropoxy)-methyl ester (2.0 g) is treated with trifluoroacetic acid (approximately 3 ml) and stirred at room temperature for fifteen minutes. The trifluoroacetic acid is removed in vacuo and the residue is taken up in tetrahydrofuran and added dropwise to the above mixture over a thirty minute period at room temperature. After sixteen hours the tetrahydrofuran is stripped and the residue is parti-

tioned between 5% potassium bisulfate and ether. The layers are separated and the organic portion is washed with 5% monobasic sodium phosphate (three times), brine, and dried ($MgSO_4$). The solvent is stripped to yield a solid (2.6 g). Trituration of the crude solid with ether followed by filtration yields crystals (1.8 g), melting point 124°-125° C.

EXAMPLE 50

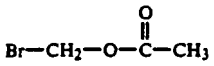
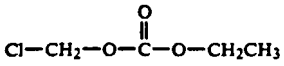
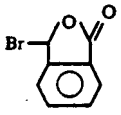
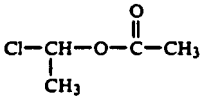
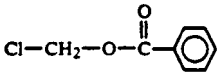
(cis)-4-Cyclohexyl-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)-methyl ester, lithium salt

(cis)-4-Cyclohexyl-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)-methyl ester (1.21 g) is dissolved in acetone. Lithium carbonate (0.082 g) and water are added to the stirring solution. After three hours some of the acetone is stripped (due to soap-like solution no more acetone can be removed). The remaining solution is lyophilized. The lyophilizate is then redissolved in water, Millipore filtered, and relyophilized. A fluffy solid is obtained (1.1 g).

Anal. Calc'd. for $C_{29}H_{43}NO_7P-Li^+.0.5$ mole of H_2O: H, 2.47; C, 61.58; N, 7.84; P, 5.5. Found: H, 2.42; C, 61.43; N, 7.75; P, 5.3.

EXAMPLES 51-55

Following the procedure of example 42, but substituting the alkylating agent in column I for chloromethyl-pivalate, yields the compound listed in column II.

	Column I	Column II
51.		1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,(1-oxoethoxy)methyl ester
52.		1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,ethoxycarbonyloxymethyl ester
53.		1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,phthalide ester
54.		1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,1-(1-oxoethoxy)ethyl ester
55.		1-[[ethoxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline,benzoyloxymethyl ester

EXAMPLE 56

(cis)-[[Hydroxy(phenylbutyl)phosphinyl]acetyl]-L-proline,(2,2-dimethyl-1-oxopropoxy)methyl ester

(A) (cis)-4-Phenyl-1-phenylmethoxycarbonyl-L-proline

A slurry of (cis)-4-phenyl-L-proline, hydrochloride (3.0 g) in 10 ml of water is cooled in an ice-bath and treated dropwise with a solution of 1.01 g of sodium hydroxide in 10 ml of water. The resulting suspension is treated simultaneously dropwise with benzyl chloroformate (2.28 g) and a solution of 0.5 g of sodium hydroxide in 5 ml of water. Additional water is added to a total volume of about 100 ml. (Vigorous shaking of the flask is needed to insure complete mixing.) After 2 hours, the solids are filtered, washed with water, washed three times with 1:1 ether:acetone, washed with ether and air dried for about 16 hours to give 4.2 g of the sodium salt of the title compound.

The filtrates are combined, concentrated in vacuo and washed with ether. The aqueous layers are acidified with concentrated hydrochloric acid and extracted twice with dichloromethane. The organic layers are washed with saturated brine, dried ($MgSO_4$) and concentrated in vacuo to give 0.4 g of the title compound.

The above 4.2 g of sodium salt is partitioned between dichloromethane (twice) and 25 ml of N hydrochloric acid. The organic layers are washed with saturated brine, dried ($MgSO_4$) and concentrated in vacuo to give 3.17 g of the title compound.

(B)
(cis)-4-Phenyl-1-phenylmethoxycarbonyl-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

(cis)-4-Phenyl-1-phenylmethoxycarbonyl-L-proline (3.52 g), dissolved in 25 ml of dry dimethylformamide, is treated with 1.61 g of anhydrous potassium fluoride and 2.0 g of chloromethyl pivalate under argon at room temperature for about 16 hours. The reaction mixture is diluted with ethyl acetate and washed with water (three times), saturated sodium bicarbonate and saturated brine. The aqueous layers are backwashed with fresh ethyl acetate. The combined organic layers are dried ($MgSO_4$) and concentrated in vacuo to give 4.86 g of an oil.

(C) (cis)-4-Phenyl-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, p-toluenesulfonic acid salt

A solution of 4.86 g of (cis)-4-phenyl-1-(phenylmethoxycarbonyl)-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester and 2.31 g of toluenesulfonic acid monohydrate in 75 ml of ethyl acetate containing 0.5 g of 10% palladium on charcoal is shaken on a Parr hydrogenator at an initial pressure of 35 psi of hydrogen for 45 minutes (final pressure is 26 psi). The precipitated solids are dissolved with dichloromethane and the catalyst is filtered through Celite. The filtrate is concentrated in vacuo and the solid residue is triturated with 100 ml of ethyl acetate, filtered and washed with ether to give 4.14 g of the title compound, melting point 167°-170° C.

(D)
(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]-acetyl]-4-phenyl-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester

A solution of 1.85 g of [hydroxy(4-phenylbutyl)phosphinyl]acetic acid in 50 ml of dry tetrahydrofuran at 0°-5° C. under argon is treated with 1.17 g of carbonyldiimidazole. After stirring at 0°-5° C. for 1 hour, 2.02 ml of triethylamine is added followed by the portionwise addition of (cis)-4-phenyl-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, p-toluene-sulfonic acid salt (4.14 g) over a 20 minute period. An additional 50 ml of tetrahydrofuran is added and the mixture is allowed to stir at room temperature for about 16 hours. Solvent is then removed in vacuo and the residue, dissolved in ethyl acetate, is washed with 10% potassium bisulfate, 5% monobasic sodium phosphate (three times) and saturated brine. The organic layer is dried ($MgSO_4$) and concentrated in vacuo to give 5 g of crude product. Chromatography of 2.9 g of crude on 70 g of silica gel eluted (i) with 3.5–5% methanol/dichloromethane yields 1.5 g of a mixture (ii) with 7.5% methanol/dichloromethane yields 0.52 g of homogeneous product. Rechromatography of the mixture gives an additional 0.51 g of homogeneous product.

Anal. Calc'd. $C_{29}H_{38}NPO_7.1.5H_2O$: C, 61.04; H, 7.24; N, 2.45. Found: C, 60.75; H, 6.54; N, 2.53.

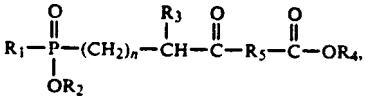
EXAMPLE 57

(cis)-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-phenyl-L-proline

A solution of (cis)-1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-phenyl-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester (300 mg) is dissolved in 10 ml of 50% aqueous ethanol and treated with 1.5 ml of 1 N sodium hydroxide at room temperature for about 16 hours. The mixture is diluted with 15 ml of water and washed with 4:1 ether/hexane. The organic layer is washed with two fresh 10 ml portions of water. The combined aqueous layer is acidified with 1 N hydrochloric acid and extracted with two 50 ml portions of ethyl acetate. The organic layer is washed with saturated brine, dried ($MgSO_4$) and concentrated in vacuo to give 150 mg of material. Trituration with ether yields 100 mg of product; melting point softening starts at 80° C., clear at 120° C., after drying in vacuo over phosphorous pentoxide for 5 hours.

What is claimed is:

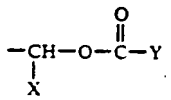
1. A compound having the formula



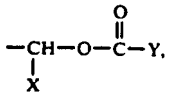
or a salt thereof, wherein

R_1 is alkyl, aryl, arylalkyl, cycloalkyl, or cycloalkylalkyl;

one of R_2 and R_4 is



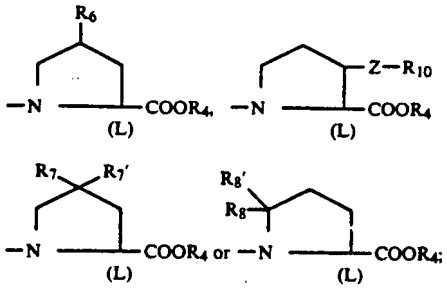
and the other is hydrogen, alkyl, arylalkyl or



wherein X is hydrogen, alkyl or phenyl and Y is hydrogen, alkyl, phenyl or alkoxy, or together X and Y are $-(CH_2)_2-$, $-(CH_2)_3-$, $-CH=CH-$ or

R_3 is hydrogen or alkyl;

$-R_5-COOR_4$ is



R_6 is hydrogen, hydroxy, alkyl, halogen, azido, amino, cycloalkyl, aryl, arylalkyl, carbamoyloxy, N,N-dialkylcarbamoyloxy, or $-Z-R_9$;

R_7 and R_7' are the same and each is halogen or $-Z-R_{10}$, or R_7 and R_7' together are $=O$, $-O-(CH_2)_m-O-$ or $-S-(CH_2)_m-S-$;

R_8 is hydrogen and R_8' is phenyl, 2-hydroxyphenyl or 4-hydroxyphenyl or R_8 and R_8' together are $=O$;

R_9 is alkyl, aryl, arylalkyl, 1- or 2-naphthyl, or biphenyl;

R_{10} is alkyl, aryl or arylalkyl;

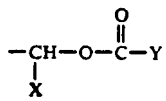
Z is oxygen or sulfur;

n is 0 or 1; and

m is 1 or 2;

and wherein the term "aryl" refers to phenyl or phenyl substituted with halogen, alkyl, alkoxy, alkylthio, hydroxy, alkanoyl, nitro, amino, dialkylamino or trifluoromethyl groups; the term "alkyl" refers to groups having 1 to 10 carbon atoms; the term "alkoxy" refers to groups having 1 to 8 carbon atoms; the term "cycloalkyl" refers to groups having 3 to 7 carbon atoms; and the term "alkanoyl" refers to groups having 2 to 9 carbon atoms.

2. A compound in accordance with claim 1 wherein one of R_2 and R_4 is

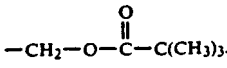


and the other is hydrogen.

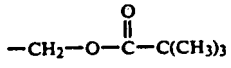
3. A compound in accordance with claim 4 wherein R_1 is 4-phenylbutyl and R_3 is hydrogen.

4. A compound in accordance with claim 1 wherein n is 0.

5. A compound in accordance with claim 2 wherein R_2 is hydrogen and R_4 is

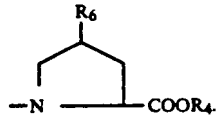


6. A compound in accordance with claim 2 wherein R_2 is



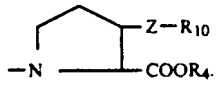
and R_4 is hydrogen.

7. A compound in accordance with claim 1 wherein $-R_5-COOR_4$ is



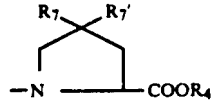
(L)

8. A compound in accordance with claim 1 wherein $-R_5-COOR_4$ is



(L)

9. A compound in accordance with claim 1 wherein $-R_5-COOR_4$ is



(L)

10. A compound in accordance with claim 1 wherein $-R_5-COOR_4$ is

(L)

11. The compound in accordance with claim 1, (S)-7-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid, (2,2-dimethyl-1-oxopropoxy)methyl ester, or a salt thereof.

12. The compound in accordance with claim 1, 1-[[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline, (2,2-dimethyl-1-oxopropoxy)methyl ester, or a salt thereof.

13. The compound in accordance with claim 1, 1-[[[(2,2-dimethyl-1-oxopropoxy)methoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline, or a salt thereof.

* * * * *

Mead Johnson
PHARMACEUTICALS

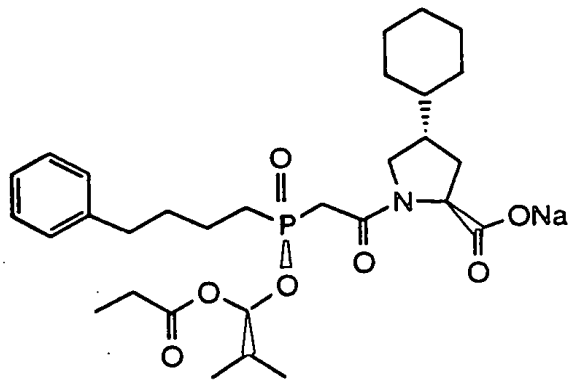
J4-412A

MONOPRIL®
Fosinopril Sodium Tablets

DESCRIPTION
MONOPRIL (Fosinopril Sodium) is the sodium salt of fosinopril, the ester prodrug of an angiotensin converting enzyme (ACE) inhibitor, fosinoprilat. It contains a phosphinate group capable of specific binding to the active site of angiotensin converting enzyme. Fosinopril sodium is designated chemically as: L-proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy) propoxy] (4-phenylbutyl) phosphinyl]acetyl]-, sodium salt, *trans*-.

Fosinopril sodium is a white to off-white crystalline powder. It is soluble in water (100 mg/mL), methanol, and ethanol and slightly soluble in hexane.

Its structural formula is:



Its empiric formula is $C_{30}H_{45}NNaO_7P$, and its molecular weight is 585.65.

MONOPRIL is available for oral administration as 10 mg and 20 mg tablets. Inactive ingredients include: lactose, microcrystalline cellulose, crospovidone, povidone, and magnesium stearate.

CLINICAL PHARMACOLOGY
Mechanism of Action
In animals and humans, fosinopril sodium is hydrolyzed by esterases to the pharmacologically active form, fosinoprilat, a specific competitive inhibitor of angiotensin converting enzyme (ACE).

ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decreased aldosterone secretion. The latter decrease may result in a small increase of serum potassium.

In 647 hypertensive patients treated with fosinopril alone for an average of 29 weeks, mean increases in serum potassium of 0.1 mEq/L were observed. Similar increases were observed among all patients treated with fosinopril, including those receiving concomitant diuretic therapy. Removal of angiotensin II negative feedback on renin secretion leads to increased plasma renin activity.

ACE is identical to kininase, an enzyme that degrades bradykinin. Whether increased levels of bradykinin, a potent vasodepressor peptide, play a role in the therapeutic effects of MONOPRIL remains to be elucidated.

While the mechanism through which MONOPRIL lowers blood pressure is believed to be primarily suppression of the renin-angiotensin-aldosterone system, MONOPRIL has an antihypertensive effect even in patients with low-renin hypertension. Although MONOPRIL was antihypertensive in all races studied, black hypertensive patients (usually a low-renin hypertensive population) had a smaller average response to ACE inhibitor monotherapy than non-black patients.

Pharmacokinetics and Metabolism
Following oral administration, fosinopril (the prodrug) is absorbed slowly. The absolute absorption of fosinopril averaged 36% of an oral dose. The primary site of absorption is the proximal small intestine (duodenum/jejunum). While the rate of absorption may be slowed by the presence of food in the gastrointestinal tract, the extent of absorption of fosinopril is essentially unaffected.

Fosinoprilat is highly protein-bound (≥ 95%), has a relatively small volume of distribution, and has negligible binding to cellular components in blood. After single and multiple oral doses, plasma levels, areas under plasma concentration-time curves (AUCs) and peak concentrations (Cmaxs) are directly proportional to the dose of fosinopril. Times to peak concentrations are independent of dose and are achieved in approximately 3 hours.

After an oral dose of radiolabeled fosinopril, 75% of radioactivity in plasma was present as active fosinoprilat, 20-30% as a glucuronide conjugate of fosinoprilat, and 1-5% as a *p*-hydroxy metabolite of fosinoprilat. Since fosinoprilat is not biotransformed after intravenous administration, fosinopril, not fosinoprilat, appears to be the precursor for the glucuronide and *p*-hydroxy metabolites. In rats, the *p*-hydroxy metabolite of fosinoprilat is as potent an inhibitor of ACE as fosinoprilat; the glucuronide conjugate is devoid of ACE inhibitory activity.

After intravenous administration, fosinoprilat was eliminated approximately equally by the liver and kidney. After oral administration of radiolabeled fosinopril, approximately half of the absorbed dose is excreted in the urine and the remainder is excreted in the feces. In two studies involving healthy subjects, the mean body clearance of intravenous fosinoprilat was between 26 and 39 mL/min.

In healthy subjects, the terminal elimination half-life ($t_{1/2}$) of an intravenous dose of radiolabeled fosinoprilat is approximately 12 hours. In hypertensive patients with normal renal and hepatic function, who received repeated doses of fosinopril, the effective $t_{1/2}$ for accumulation of fosinoprilat averaged 11.5 hours.

In patients with renal insufficiency (creatinine clearance <80 mL/min/1.73m²), the total body clearance of fosinoprilat is approximately one-half of that in patients with normal renal function, while absorption, bioavailability, and protein-binding are not appreciably altered. The clearance of fosinoprilat does not differ appreciably with degree of renal insufficiency, because the diminished renal elimination is offset by increased hepatobiliary elimination. A modest increase in plasma AUC levels (less than two times that in normals) was observed in patients with various degrees of renal insufficiency, including end-stage renal failure (creatinine clearance <10 mL/min/1.73m²). (See DOSAGE AND ADMINISTRATION.)

Fosinopril is not well dialyzed. Clearance of fosinoprilat by hemodialysis and peritoneal dialysis averages 2% and 7%, respectively, of urea clearances.

In patients with hepatic insufficiency (alcoholic or biliary cirrhosis), the extent of hydrolysis of fosinopril is not appreciably reduced, although the rate of hydrolysis may be slowed; the apparent total body clearance of fosinoprilat is approximately one-half of that in patients with normal hepatic function.

In elderly (male) subjects (65-74 years old) with clinically normal renal and hepatic function, there appear to be no significant differences in pharmacokinetic parameters for fosinoprilat compared to those of younger subjects (20-35 years old).

Fosinoprilat was found to cross the placenta of pregnant animals.

Studies in animals indicate that fosinopril and fosinoprilat do not cross the blood-brain barrier.

Pharmacodynamics and Clinical Effects
Serum ACE activity was inhibited by ≥ 90% at 2 to 12 hours after single doses of 10 to 40 mg of fosinopril. At 24 hours, serum ACE activity remained suppressed by 85%, 93%, and 93% in the 10, 20, and 40 mg dose groups, respectively.

Administration of MONOPRIL (Fosinopril Sodium) to patients with mild to moderate hypertension results in a reduction of both supine and standing blood pressure to about the same extent with no compensatory tachycardia. Symptomatic postural hypotension is infrequent, although it can occur in patients who are salt- and/or volume-depleted (see WARNINGS). Use of MONOPRIL in combination with thiazide diuretics gives a blood pressure-lowering effect greater than that seen with either agent alone.

Following oral administration of single doses of 10-40 mg, MONOPRIL lowered blood pressure within one hour, with peak reductions achieved 2-6 hours after dosing. The antihypertensive effect of a single dose persisted for 24 hours. Following four weeks of monotherapy in placebo-controlled trials in patients with mild to moderate hypertension, once daily doses of 20-80 mg lowered supine or seated systolic and diastolic blood pressures 24 hours after

dosing by an average of 8-9/6-7 mmHg more than placebo. The trough effect was about 50-60% of the peak diastolic response and about 80% of the peak systolic response.

In most trials, the antihypertensive effect of MONOPRIL (Fosinopril Sodium) increased during the first several weeks of repeated measurements. The antihypertensive effect of MONOPRIL has been shown to continue during long-term therapy for at least 2 years. Abrupt withdrawal of MONOPRIL has not resulted in a rapid increase in blood pressure.

Limited experience in controlled and uncontrolled trials combining fosinopril with a calcium channel blocker or a loop diuretic has indicated no unusual drug-drug interactions. Other ACE inhibitors have had less than additive effects with beta-adrenergic blockers, presumably because both drugs lower blood pressure by inhibiting parts of the renin-angiotensin system.

ACE inhibitors are generally less effective in blacks than in non-blacks. The effectiveness of MONOPRIL was not influenced by age, sex, or weight.

In hemodynamic studies in hypertensive patients, after three months of therapy, responses (changes in BP, heart rate, cardiac index, and PVR) to various stimuli (e.g., isometric exercise, 45° head-up tilt, and mental challenge) were unchanged compared to baseline, suggesting that MONOPRIL does not affect the activity of the sympathetic nervous system. Reduction in systemic blood pressure appears to have been mediated by a decrease in peripheral vascular resistance without reflex cardiac effects. Similarly, renal, splanchnic, cerebral, and skeletal muscle blood flow were unchanged compared to baseline, as was glomerular filtration rate.

INDICATIONS AND USAGE

MONOPRIL is indicated for the treatment of hypertension. It may be used alone or in combination with thiazide diuretics.

In using MONOPRIL, consideration should be given to the fact that another angiotensin converting enzyme inhibitor, captopril, has caused agranulocytosis, particularly in patients with renal impairment or collagen-vascular disease. Available data are insufficient to show that MONOPRIL does not have a similar risk (see WARNINGS).

CONTRAINDICATIONS

MONOPRIL is contraindicated in patients who are hypersensitive to this product or to any other angiotensin converting enzyme inhibitor (e.g., a patient who has experienced angioedema with any other ACE inhibitor therapy).

WARNINGS

Angioedema

Angioedema involving the extremities, face, lips, mucous membranes, tongue, glottis or larynx has been reported in patients treated with ACE inhibitors. If angioedema involves the tongue, glottis or larynx, airway obstruction may occur and be fatal. If laryngeal stridor or angioedema of the face, lips, mucous membranes, tongue, glottis or extremities occurs, treatment with MONOPRIL should be discontinued and appropriate therapy instituted immediately. **Where there is involvement of the tongue, glottis, or larynx, likely to cause airway obstruction, appropriate therapy, e.g., subcutaneous epinephrine solution 1:1000 (0.3 mL to 0.5 mL) should be promptly administered** (see PRECAUTIONS: Information for Patients and ADVERSE REACTIONS).

Hypotension

MONOPRIL can cause symptomatic hypotension. Like other ACE inhibitors, fosinopril has been only rarely associated with hypotension in uncomplicated hypertensive patients. Symptomatic hypotension is most likely to occur in patients who have been volume- and/or salt-depleted as a result of prolonged diuretic therapy, dietary salt restriction, dialysis, diarrhea, or vomiting. Volume and/or salt depletion should be corrected before initiating therapy with MONOPRIL.

In patients with congestive heart failure, with or without associated renal insufficiency, ACE inhibitor therapy may cause excessive hypotension, which may be associated with oliguria or azotemia and, rarely, with acute renal failure and death. In such patients, MONOPRIL therapy should be started under close medical supervision; they should be followed closely for the first 2 weeks of treatment and whenever the dose of fosinopril or diuretic is increased.

If hypotension occurs, the patient should be placed in a supine position, and, if necessary, treated with intravenous infusion of physiological saline. MONOPRIL treatment usually can be continued following restoration of blood pressure and volume.

Neutropenia/Agranulocytosis

Another angiotensin converting enzyme inhibitor, captopril, has been shown to cause agranulocytosis and bone marrow depression, rarely in uncomplicated patients, but more frequently in patients with renal impairment, especially if they also have a collagen-vascular disease such as systemic lupus erythematosus or scleroderma. Available data from clinical trials of fosinopril are insufficient to show that fosinopril does not cause agranulocytosis at similar rates. Monitoring of white blood cell counts should be considered in patients with collagen-vascular disease, especially if the disease is associated with impaired renal function.

Fetal/Neonatal Morbidity and Mortality

ACE inhibitors can cause fetal and neonatal morbidity and mortality when administered to pregnant women.

When ACE inhibitors have been used during the second and third trimesters of pregnancy, there have been reports of neonatal hypotension, renal failure, skull hypoplasia, and death. Oligohydramnios has also been reported, presumably resulting from decreased fetal renal function; oligohydramnios has been associated with fetal limb contractures, craniofacial malformations, hypoplastic lung development, and intrauterine growth retardation. Prematurity and patent ductus arteriosus have been reported, although it is not clear whether these occurrences were due to the ACE inhibitor exposure or to the mother's underlying disease.

It is not known whether exposure limited to the first trimester can adversely affect fetal outcome.

A patient who becomes pregnant while taking ACE inhibitors, or who takes ACE inhibitors when already pregnant, should be apprised of the potential hazard to her fetus. If she continues to receive ACE inhibitors during the second or third trimester of pregnancy, frequent ultrasound examinations should be performed to look for oligohydramnios. When oligohydramnios is found, ACE inhibitors should generally be discontinued.

Infants with histories of *in utero* exposure to ACE inhibitors should be closely observed for hypotension, oliguria, and hyperkalemia. If oliguria occurs, attention should be directed toward support of blood pressure and renal perfusion.

Fosinopril is poorly dialyzed from the circulation of adults by hemodialysis and peritoneal dialysis. There is no experience with any procedure for removing fosinopril from the neonatal circulation.

In pregnant rabbits, maternal toxicity was evident at doses ranging from 2.5 to 40 mg/kg/day (approximately 3 to 50 times the maximum recommended human dose). Fosinopril was embryocidal in rabbits at 10 and 40 mg/kg/day (approximately 12 and 50 times the maximum recommended human dose). These effects were probably due to marked decreases in blood pressure caused by ACE inhibition in this species. There were no teratogenic effects in rabbits at any dose level tested.

In pregnant rats, there was evidence of maternal toxicity at all dose levels tested, i.e., 25 to 400 mg/kg/day (about 30 to 500 times the maximum recommended human dose). Slight reductions in placental weights and degree of skeletal ossification were observed at all dose levels, and fetal body weights were reduced in the high-dose group. Three similar orofacial malformations and one fetus with *situs inversus* occurred in fosinopril-treated animals. The association of these anomalies with treatment is uncertain.

PRECAUTIONS

General

Impaired Renal Function: As a consequence of inhibiting the renin-angiotensin-aldosterone system, changes in renal function may be anticipated in susceptible individuals. In patients with severe congestive heart failure whose renal function may depend on the activity of the renin-angiotensin-aldosterone system, treatment with angiotensin converting enzyme inhibitors, including MONOPRIL (Fosinopril Sodium), may be associated with oliguria and/or progressive azotemia and (rarely) with acute renal failure and/or death.

In hypertensive patients with renal artery stenosis in a solitary kidney or bilateral renal artery stenosis, increases in blood urea nitrogen and serum creatinine may occur. Experience with another angiotensin converting enzyme inhibitor suggests that these increases are usually reversible upon discontinuation of ACE inhibitor and/or diuretic therapy. In such patients, renal function should be monitored during the first few weeks of therapy. Some hypertensive patients with no apparent pre-existing renal vascular disease have developed increases in blood urea nitrogen and serum creatinine, usually minor and transient, especially when MONOPRIL has been given concomitantly with a diuretic. This is more likely to occur in patients with pre-existing renal impairment. Dosage reduction of MONOPRIL and/or discontinuation of the diuretic may be required.

Evaluation of the hypertensive patient should always include assessment of renal function (see DOSAGE AND ADMINISTRATION).

Impaired renal function decreases total clearance of fosinoprilat and approximately doubles AUC. In general, however, no adjustment of dosing is needed (see CLINICAL PHARMACOLOGY).

Hyperkalemia: In clinical trials, hyperkalemia (serum potassium greater than 10% above the upper limit of normal) has occurred in approximately 2.6% of hypertensive patients receiving MONOPRIL. In most cases, these were isolated values which resolved despite continued therapy. In clinical trials, 0.1% of patients (two patients) were discontinued from therapy due to an elevated serum potassium. Risk factors for the development of hyperkalemia include renal insufficiency, diabetes mellitus, and the concomitant use of

potassium-sparing diuretics, potassium supplements, and/or potassium-containing salt substitutes, which should be used cautiously, if at all, with MONOPRIL (Fosinopril Sodium) (see PRECAUTIONS: Drug Interactions).

Impaired Liver Function: Since fosinopril is primarily metabolized by hepatic and gut wall esterases to its active moiety, fosinoprilat, patients with impaired liver function could develop elevated plasma levels of unchanged fosinopril. In a study in patients with alcoholic or biliary cirrhosis, the extent of hydrolysis was unaffected, although the rate was slowed. In these patients, the apparent total body clearance of fosinoprilat was decreased and the plasma AUC approximately doubled.

Surgery/Anesthesia: In patients undergoing surgery or during anesthesia with agents that produce hypotension, fosinopril will block the angiotensin II formation that could otherwise occur secondary to compensatory renin release. Hypotension that occurs as a result of this mechanism can be corrected by volume expansion.

Information for Patients

Angioedema: Angioedema, including laryngeal edema, can occur with treatment with ACE inhibitors, especially following the first dose. Patients should be advised to immediately report to their physician any signs or symptoms suggesting angioedema (e.g., swelling of face, eyes, lips, tongue, larynx, mucous membranes, and extremities; difficulty in swallowing or breathing; hoarseness) and to discontinue therapy. (See WARNINGS and ADVERSE REACTIONS.)

Symptomatic Hypotension: Patients should be cautioned that lightheadedness can occur, especially during the first days of therapy, and it should be reported to a physician. Patients should be told that if syncope occurs, MONOPRIL should be discontinued until the physician has been consulted.

All patients should be cautioned that inadequate fluid intake or excessive perspiration, diarrhea, or vomiting can lead to an excessive fall in blood pressure, with the same consequences of lightheadedness and possible syncope.

Hyperkalemia: Patients should be told not to use potassium supplements or salt substitutes containing potassium without consulting the physician.

Neutropenia: Patients should be told to promptly report any indication of infection (e.g., sore throat, fever), which could be a sign of neutropenia.

Drug Interactions

With diuretics: Patients on diuretics, especially those with intravascular volume depletion, may occasionally experience an excessive reduction of blood pressure after initiation of therapy with MONOPRIL. The possibility of hypotensive effects with MONOPRIL can be minimized by either discontinuing the diuretic or increasing salt intake prior to initiation of treatment with MONOPRIL. If this is not possible, the starting dose should be reduced and the patient should be observed closely for several hours following an initial dose and until blood pressure has stabilized (see DOSAGE AND ADMINISTRATION).

With potassium supplements and potassium-sparing diuretics: MONOPRIL can attenuate potassium loss caused by thiazide diuretics. Potassium-sparing diuretics (spironolactone, amiloride, triamterene, and others) or potassium supplements can increase the risk of hyperkalemia. Therefore, if concomitant use of such agents is indicated, they should be given with caution, and the patient's serum potassium should be monitored frequently.

With lithium: Increased serum lithium levels and symptoms of lithium toxicity have been reported in patients receiving ACE inhibitors during therapy with lithium. These drugs should be coadministered with caution, and frequent monitoring of serum lithium levels is recommended. If a diuretic is also used, the risk of lithium toxicity may be increased.

With antacids: In a clinical pharmacology study, coadministration of an antacid (aluminum hydroxide, magnesium hydroxide, and simethicone) with fosinopril reduced serum levels and urinary excretion of fosinoprilat as compared with fosinopril administrated alone, suggesting that antacids may impair absorption of fosinopril. Therefore, if concomitant administration of these agents is indicated, dosing should be separated by 2 hours.

Other: Neither MONOPRIL nor its metabolites have been found to interact with food. In separate single or multiple dose pharmacokinetic interaction studies with chlorthalidone, nifedipine, propranolol, hydrochlorothiazide, cimetidine, metoclopramide, propantheline, digoxin, and warfarin, the bioavailability of fosinoprilat was not altered by coadministration of fosinopril with any one of these drugs. In a study with concomitant administration of aspirin and MONOPRIL, the bioavailability of unbound fosinoprilat was not altered.

In a pharmacokinetic interaction study with warfarin, bioavailability parameters, the degree of protein binding, and the anticoagulant effect (measured by prothrombin time) of warfarin were not significantly changed.

Drug/Laboratory Test Interaction

Fosinopril may cause a false low measurement of serum digoxin levels with the Digi-Tab® RIA Kit for Digoxin. Other kits, such as the Coat-A-Count® RIA Kit, may be used.

Carcinogenesis, Mutagenesis, and Impairment of Fertility

No evidence of a carcinogenic effect was found when fosinopril was given in the diet to mice and rats for up to 24 months at doses up to 400 mg/kg/day. On a body weight basis, the highest dose in mice and rats is about 250 times the maximum human dose of 80 mg, assuming a 50 kg subject. On a body surface area basis, in mice, this dose is 20 times the maximum human dose; in rats, this dose is 40 times the maximum human dose. Male rats given the highest dose level had a slightly higher incidence of mesentery/omentum lipomas.

Neither fosinopril nor the active fosinoprilat was mutagenic in the Ames microbial mutagen test, the mouse lymphoma forward mutation assay, or a mitotic gene conversion assay. Fosinopril was also not genotoxic in a mouse micronucleus test *in vivo* and a mouse bone marrow cytogenetic assay *in vivo*.

In the Chinese hamster ovary cell cytogenetic assay, fosinopril increased the frequency of chromosomal aberrations when tested without metabolic activation at a concentration that was toxic to the cells. However, there was no increase in chromosomal aberrations at lower drug concentrations without metabolic activation or at any concentration with metabolic activation.

There were no adverse reproductive effects in male and female rats treated with 15 or 60 mg/kg daily. On a body weight basis, the high dose of 60 mg/kg is about 38 times the maximum recommended human dose. On a body surface area basis, this dose is 6 times the maximum recommended human dose. There was no effect on pairing time prior to mating in rats until a daily dose of 240 mg/kg, a toxic dose, was given; at this dose, a slight increase in pairing time was observed. On a body weight basis, this dose is 150 times the maximum recommended human dose. On a body surface area basis, this dose is 24 times the maximum recommended human dose.

Pregnancy

Pregnancy Category D: See WARNINGS: Fetal/Neonatal Morbidity and Mortality.

Nursing Mothers

Ingestion of 20 mg daily for three days resulted in detectable levels of fosinoprilat in breast milk. MONOPRIL (Fosinopril Sodium) should not be administered to nursing mothers.

Geriatric Use

Of the total number of patients who received fosinopril in US clinical studies of MONOPRIL, 13% were 65 and older while 1.3% were 75 and older. No overall differences in effectiveness or safety were observed between these patients and younger patients, and other reported clinical experience has not identified differences in response between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

In a pharmacokinetic study comparing elderly (65-74 years old) and nonelderly (20-35 years old) healthy volunteers, there were no differences between the groups in peak fosinoprilat levels or area under the plasma concentration time curve (AUC).

Pediatric Use

Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

MONOPRIL (Fosinopril Sodium) has been evaluated for safety in more than 1500 individuals in hypertension trials, including approximately 450 patients treated for a year or more. Generally adverse events were mild and transient, and their frequency was not related to dose within the recommended daily dosage range.

In placebo-controlled clinical trials (688 fosinopril-treated patients), the usual duration of therapy was two to three months. Discontinuations due to any clinical or laboratory adverse event were 4.1 and 1.1 percent in fosinopril-treated and placebo-treated patients, respectively. The most frequent reasons (0.4 to 0.9%) were headache, elevated transaminases, fatigue, cough, diarrhea, and nausea and vomiting.

During clinical trials with any fosinopril regimen, the incidence of adverse events in the elderly (≥65 years old) was similar to that seen in younger patients.

Clinical adverse events probably or possibly related or of uncertain relationship to therapy, occurring in at least 1% of patients treated with MONOPRIL alone in placebo-controlled clinical trials are shown in the table below.

Clinical Adverse Events in Placebo-Controlled Trials

	MONOPRIL (N = 688) Incidence (Discontinuation)	Placebo (N = 184) Incidence (Discontinuation)
Headache	3.2 (0.9)	3.3
Cough	2.2 (0.4)	0.0
Dizziness	1.6	0.0
Diarrhea	1.5 (0.4)	1.6
Fatigue	1.5 (0.6)	1.6
Nausea/Vomiting	1.2 (0.4)	0.5
Sexual Dysfunction	1.0 (0.1)	1.1 (0.5)

Other clinical events probably or possibly related, or of uncertain relationship to therapy occurring in 0.2 to 1.0% of patients (except as noted) treated with MONOPRIL in controlled or uncontrolled clinical trials (N = 1479) and less frequent, clinically significant events include (listed by body system):

General: Chest pain, edema, weakness, excessive sweating.

Cardiovascular: Angina/myocardial infarction, cerebrovascular accident, hypertensive crisis, rhythm disturbances, palpitations, hypotension, syncope, flushing, claudication.

Orthostatic hypotension occurred in 1.4% of patients treated with fosinopril monotherapy. Hypotension or orthostatic hypotension was a cause for discontinuation of therapy in 0.1% of patients.

Dermatologic: Urticaria, rash, photosensitivity, pruritus.
Endocrine/Metabolic: Gout, decreased libido.
Gastrointestinal: Pancreatitis, hepatitis, dysphagia, abdominal distention, abdominal pain, flatulence, constipation, heartburn, appetite/weight change, dry mouth.
Hematologic: Lymphadenopathy.
Immunologic: Angioedema.
Musculoskeletal: Arthralgia, musculoskeletal pain, myalgia/muscle cramp.
Nervous/Psychiatric: Memory disturbance, tremor, confusion, mood change, paresthesia, sleep disturbance, drowsiness, vertigo.
Respiratory: Bronchospasm, pharyngitis, sinusitis/rhinitis, laryngitis/hoarseness, epistaxis. A symptom-complex of cough, bronchospasm, and eosinophilia has been observed in two patients treated with fosinopril.
Special Senses: Tinnitus, vision disturbance, taste disturbance, eye irritation.
Urogenital: Renal insufficiency, urinary frequency.

Potential Adverse Effects Reported with ACE Inhibitors
Other medically important adverse effects reported with ACE inhibitors include: Cardiac arrest; eosinophilic pneumonitis; neutropenia/agranulocytosis, pancytopenia, anemia (including hemolytic and aplastic), thrombocytopenia; acute renal failure; hepatic failure, jaundice (hepatocellular or cholestatic); symptomatic hyponatremia; bullous pemphigus, exfoliative dermatitis; a syndrome which may include: arthralgia/arthritis, vasculitis, serositis, myalgia, fever, rash or other dermatologic manifestations, a positive ANA, leukocytosis, eosinophilia, or an elevated ESR.

Laboratory Test Abnormalities
Serum Electrolytes: Hyperkalemia, (see PRECAUTIONS); *hyponatremia,* (see PRECAUTIONS: Drug Interactions, With diuretics).
BUN/Serum Creatinine: Elevations, usually transient and minor, of BUN or serum creatinine have been observed. In placebo-controlled clinical trials, there were no significant differences in the number of patients experiencing increases in serum creatinine (outside the normal range or 1.33 times the pre-treatment value) between the fosinopril and placebo treatment groups. Rapid reduction of longstanding or markedly elevated blood pressure by any antihypertensive therapy can result in decreases in the glomerular filtration rate and, in turn, lead to increases in BUN or serum creatinine. (See PRECAUTIONS: General.)
Hematology: In controlled trials, a mean *hemoglobin* decrease of 0.1 g/dL was observed in fosinopril-treated patients. In individual patients decreases in hemoglobin or hematocrit were usually transient, small, and not associated with symptoms. No patient was discontinued from therapy due to the development of anemia. *Other:* Neutropenia (see WARNINGS), leukopenia and eosinophilia.
Liver Function Tests: Elevations of transaminases, LDH, alkaline phosphatase and serum bilirubin have been reported. Fosinopril therapy was discontinued because of serum transaminase elevations in 0.7% of patients. In the majority of cases, the abnormalities were either present at baseline or were associated with other etiologic factors. In those cases which were possibly related to fosinopril therapy, the elevations were generally mild and transient and resolved after discontinuation of therapy.

OVERDOSAGE
The oral LD_{50} of fosinopril in rats is 2600 mg/kg. Human overdoses of fosinopril have not been reported, but the most common manifestation of human fosinopril overdosage is likely to be hypotension.
Laboratory determinations of serum levels of fosinoprilat and its metabolites are not widely available, and such determinations, have in any event, no established role in the management of fosinopril overdose. No data are available to suggest physiological maneuvers (e.g., maneuvers to change the pH of the urine) that might accelerate elimination of fosinopril and its metabolites. Fosinoprilat is poorly removed from the body by both hemodialysis and peritoneal dialysis.
Angiotensin II could presumably serve as a specific antagonist-antidote in the setting of fosinopril overdose, but angiotensin II is essentially unavailable outside of scattered research facilities. Because the hypotensive effect of fosinopril is achieved through vasodilation and effective hypovolemia, it is reasonable to treat fosinopril overdose by infusion of normal saline solution.

DOSAGE AND ADMINISTRATION
The recommended initial dose of MONOPRIL (Fosinopril Sodium) is 10 mg once a day, both as monotherapy and when the drug is added to a diuretic. Dosage should then be adjusted according to blood pressure response at peak (2-6 hours) and trough (about 24 hours after dosing) blood levels. The usual dosage range needed to maintain a response at trough is 20-40 mg but some patients appear to have a further response to 80 mg. In some patients treated with once daily dosing, the antihypertensive effect may diminish toward the end of the dosing interval. If trough response is inadequate, dividing the daily dose should be considered. If blood pressure is not adequately controlled with MONOPRIL alone, a diuretic may be added.
Concomitant administration of MONOPRIL with potassium supplements, potassium salt substitutes, or potassium-sparing diuretics can lead to increases of serum potassium (see PRECAUTIONS).
In patients who are currently being treated with a diuretic, symptomatic hypotension occasionally can occur following the initial dose of MONOPRIL. To reduce the likelihood of hypotension, the diuretic should, if possible, be discontinued two to three days prior to beginning therapy with MONOPRIL (see WARNINGS). Then, if blood pressure is not controlled with MONOPRIL alone, diuretic therapy should be resumed. If diuretic therapy cannot be discontinued, an initial dose of 10 mg of MONOPRIL should be used with careful medical supervision for several hours and until blood pressure has stabilized. (See WARNINGS; PRECAUTIONS: Information for Patients and Drug Interactions.)
Since concomitant administration of MONOPRIL with potassium supplements, or potassium-containing salt substitutes or potassium-sparing diuretics may lead to increases in serum potassium, they should be used with caution.
For Hypertensive Patients With Renal Impairment: In patients with impaired renal function, the total body clearance of fosinoprilat is approximately 50% slower than in patients with normal renal function. Since hepatobiliary elimination partially compensates for diminished renal elimination, the total body clearance of fosinoprilat does not differ appreciably with any degree of renal insufficiency (creatinine clearances < 80 mL/min/1.73m^2), including end-stage renal failure (creatinine clearance < 10 mL/min/1.73m^2). This relative constancy of body clearance of active fosinoprilat, resulting from the dual route of elimination, permits use of the usual dose in patients with any degree of renal impairment.

HOW SUPPLIED
10 mg tablets: White to off-white, biconvex flat-end diamond shaped, compressed tablets with unilog number **158** and **SQUIBB** on one side and M on the other. They are supplied in bottles of 100 (NDC 0087-0158-50). Bottles contain a desiccant-charcoal canister.
20 mg tablets: White to off-white, oval shaped, compressed tablets with unilog number **609** and **SQUIBB** on one side and M on the other. They are supplied in bottles of 100 (NDC 0087-0609-50). Bottles contain a desiccant-charcoal canister.
UNIMATIC® unit-dose packs containing 100 tablets are also available for each potency: **10 mg** (NDC 0087-0158-51) and **20 mg** (NDC 0087-0609-51).

STORAGE
Do not store above 86° F. Keep bottles tightly closed (protect from moisture).

CAUTION: Federal law prohibits dispensing without prescription.

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